

**US Army Corps
of Engineers®**
Engineer Research and
Development Center

Environmental Quality Technology Program

Influence of Ubiquitous Electron Acceptors on In Situ Anaerobic Biotransformation of RDX in Groundwater

Altaf H. Wani and Jeffrey L. Davis

September 2003

20031031 084

Influence of Ubiquitous Electron Acceptors on In Situ Anaerobic Biotransformation of RDX in Groundwater

Altaf H. Wani

*Applied Research Associates, Inc.
Southern Division
119 Monument Place
Vicksburg, MS 39180*

Jeffrey L. Davis

*Environmental Laboratory
U.S. Army Engineer Research and Development Center
3909 Halls Ferry Road
Vicksburg, MS 39180-6199*

Final report

Approved for public release; distribution is unlimited

Prepared for U.S. Army Corps of Engineers
 Washington, DC 20314-1000
Under EQT Work Unit AF-25

ABSTRACT: A series of column studies, with aquifer material from the former Nebraska Ordnance Plant, were performed to explore the phenomenon of electron competition from ubiquitous inorganic electron acceptors (nitrate and sulfate) present in contaminated groundwater. Acetate was used as a source of readily biodegradable carbon in all of the treatment column systems. Influent hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) concentrations (1 to 1.8 mg L⁻¹) were completely removed to below detection levels of 20 µg L⁻¹ in all treatment column systems without any nitroso-metabolites. In the control column system (with no carbon amendment), significant levels (~30 percent of the inlet molar RDX) of nitroso-substituted RDX derivates were observed in the effluent stream. The estimated first-order biodegradation rate coefficient for RDX was highest (0.79 hr⁻¹) in the treatment column system where acetate was the only amendment, about 52 times higher than the rate coefficient (0.015 hr⁻¹) obtained in the control column system. The presence of sulfate (100 mg L⁻¹) in influent groundwater temporarily delayed the onset of RDX biotransformation without any adverse effects on overall RDX biotransformation. Coexistence of low (100 mg L⁻¹) nitrate levels in the influent feed water reduced the first-order biodegradation rate coefficient obtained in the absence of nitrate by about 80 percent to 0.16 hr⁻¹. These nitrate levels, however, were low to halt the RDX biodegradation, probably because the available carbon levels were high enough to exceed the demands for nitrate reduction. High levels of nitrate (500 mg L⁻¹) initially halted RDX removal and significantly reduced the rate of RDX biotransformation by about 98 percent to 0.02 hr⁻¹, thereby increasing the half-life from 0.9 hr in the absence of nitrate to about 32 hr, with noticeable levels of untreated RDX in the effluent stream. Contrary to expectations, presence of ammonium in conjunction with acetate resulted in a lower (0.09 hr⁻¹) biodegradation rate coefficient as compared to the one obtained in absence of ammonium.

DISCLAIMER: The contents of this report are not to be used for advertising, publication, or promotional purposes. Citation of trade names does not constitute an official endorsement or approval of the use of such commercial products. All product names and trademarks cited are the property of their respective owners. The findings of this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Contents

Preface	v
1—Introduction	1
2—Literature Review	3
3—Site Description and Sampling	6
4—Materials and Methods	8
Experimental Setup	8
Operation	8
Analytical Techniques	10
Column Hydrodynamics and Biotransformation Kinetics	11
5—Results	13
Column Hydrodynamics	13
RDX Biotransformation	17
RDX Biotransformation Kinetics	22
Effects of High Nitrate Levels on RDX Biodegradation	33
6—Conclusions	36
References	37
SF 298	

List of Figures

Figure 1. Anaerobic pathway	4
Figure 2. Sampling location at NOP site	7
Figure 3. Experimental column setup	9
Figure 4. Chloride tracer breakthrough curves for individual column systems	14

Figure 5.	RDX-contaminated water flow and pH in individual column system.....	15
Figure 6.	Redox potential at column inlets and outlets in each column system.....	16
Figure 7.	Flow resistance and backpressure in individual column system.....	18
Figure 8.	RDX and nitroso-RDX intermediates concentration in influent and effluent stream.....	19
Figure 9.	Amendment concentration in column influent and effluent streams	21
Figure 10.	Axial concentration profile of RDX and its transformation products at Week 12 (Bed Profile #1).....	23
Figure 11.	Axial concentration profile of RDX and its transformation products at Week 18 (Bed Profile #2).....	24
Figure 12.	Axial concentration profile of RDX and its transformation products at Week 24 (Bed Profile #3).....	25
Figure 13.	Axial concentration profile of amendments at Week 12 (Bed Profile #1).....	27
Figure 14.	Axial concentration profile of amendments at Week 18 (Bed Profile #2).....	28
Figure 15.	Axial concentration profile of amendments at Week 24 (Bed Profile #3).....	29
Figure 16.	RDX biodegradation kinetics analysis at Week 12 (Bed Profile #1).....	30
Figure 17.	RDX biodegradation kinetics analysis at Week 18 (Bed Profile #2).....	31
Figure 18.	RDX biodegradation kinetics analysis at Week 24 (Bed Profile #3).....	32
Figure 19.	Effects of high nitrate levels on axial concentration profile of explosives, amendments, and rate of RDX biodegradation.....	34

Preface

The work reported here is the follow-on research for the biologically active zone enhancement treatability study. The purpose of this research project was to determine the influence of ubiquitous electron acceptors – nitrate and sulfate – present in groundwater on biologically mediated in situ reductive transformation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in groundwater. A series of column studies were conducted using aquifer material from the former Nebraska Ordnance Plant, Mead, NE.

The research was conducted at the Environmental Laboratory (EL), U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS. The funding for this project was provided by the Environmental Quality Technology Program, under project AF-25.

This report was prepared by Dr. Altaf H. Wani, Applied Research Associates, Inc., Vicksburg, MS; and Dr. Jeffrey L. Davis, Environmental Engineering Branch (EEB), EL. Chemical analyses were performed by the Environmental Chemistry Branch (ECB), EL. The analytical assistance provided by Ms. Anne Weathersby, ECB, throughout the research project is gratefully acknowledged.

This study was conducted under the direct supervision of Dr. Patrick N. Deliman, Chief, EEB, and Dr. Richard E. Price, Chief, Environmental Processes and Engineering Division, EL, and under the general supervision of Dr. Elizabeth C. Fleming, Acting Director, EL.

COL James R. Rowan, EN, was Commander and Executive Director of ERDC. Dr. James R. Houston was Director.

1 Introduction

Explosives and energetics in the environment represent a major concern for the U.S. Army. The most common munition-derived compounds are the nitro-aromatics such as 2,4,6-trinitrotoluene (TNT) and the nitramines such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-tetrazocine (HMX). These explosive compounds have entered the environment from sites where they were manufactured, stored, disposed, or used in military training. The Army currently has 583 sites with confirmed explosives-contaminated groundwater at 82 installations nationwide. At 22 other installations, 88 additional sites are suspected of groundwater contamination with explosives and organics (Defense Environmental Network and Information Exchange 2002).

RDX, a cyclic nitramine explosive, has contaminated soil, groundwater, and surface water at military installations, promoting concerns about potential toxic effects. There is no generally accepted in situ process currently available for the remediation of RDX in groundwater. Remediation alternatives that incorporate long-term groundwater pumping and ex situ treatment followed by discharge or reinjection of the treated water is the currently accepted remediation technology. The best available technology presently available for ex situ treatment is sorption on granular-activated carbon. Limitations of this technique are high initial capital cost for system installation; long-term operation and maintenance costs, including the high costs associated with spent carbon disposal; and the anticipated long duration of proposed remediation activities (~100 years at the former Nebraska Ordnance Plant (NOP), Mead, NE.¹

The increasing pressure for the closure of military bases throughout the United States has made identification of cost-effective and environmentally acceptable methods for remediation of explosives-contaminated groundwater an environmental imperative. Public concern over environmental contamination, combined with an increasing emphasis on the process of restoration, has led regulatory agencies to consider in situ bioremediation as a remedial alternative for cleanup of contaminated sites. The potential advantages of in situ biological treatment include low cost, ease of operation, and public acceptance. This study represents a follow-on investigation for the biologically active zone enhancement (BAZE) treatability study (Wani et al. 2002), which evaluated the potential of BAZE for in situ bioremediation of RDX using different carbon sources. The

¹ Personal Communication, 2001, T. Graff, U.S. Army Corps of Engineers (CENWK-PM-ES), Nebraska Ordnance Plant, NE.

purpose of this study was to evaluate the influence of ubiquitous electron acceptors such as nitrate and sulfate on the rate of anaerobic biotransformation of RDX under simulated in situ conditions.

2 Literature Review

Explosive contamination in soil and groundwater poses a significant cleanup challenge at many active and formerly used military sites in the United States and around the world. TNT, RDX, and HMX are the most commonly encountered energetic contaminants found at military installations. These explosives were used during World War II and are still used in various applications. In the United States, the contamination of soil and groundwater is generally attributed to ammunition production and packing in support of World War II and the Korean conflict (Pennington 1999).

RDX falls under the nitramine class of explosive compounds in which nitro groups are attached to the nitrogen atoms of a heterocyclic ring. Cyclic nitramines are widely used in explosives because of their superior explosive power (approximately 1.5 to 2 times that of TNT) and rapid detonating velocity (~1.3 times that of TNT) (U.S. Army 1984).

RDX is of particular environmental concern because studies have established that it is generally resistant to microbial transformation under aerobic conditions (McCormick et al. 1981) and it is not extensively sorbed on soils (sorption coefficient K_d of 0.83 to 0.95 L kg⁻¹) (Singh et al. 1998; Sheremata et al. 2001). Ingestion of RDX can adversely affect the central nervous system, gastrointestinal tract, and kidneys. Common symptoms of RDX intoxication include nausea, vomiting, hyperirritability, headaches, and unconsciousness (Etiner 1989). RDX also has been associated with systemic poisoning usually affecting bone marrow and the liver (Agency for Toxic Substances and Disease Registry (ATSDR) 1996). The U.S. Environmental Protection Agency (EPA) has established a drinking water health advisory of 2 $\mu\text{g L}^{-1}$ for RDX (U.S. EPA 2002). RDX can be absorbed by plants from both soil and irrigation water. In radio-labeled RDX studies, Thompson et al. (1999) and Harvey et al. (1991) observed that 60 to 86 percent of the initial radio label taken up by plants remained as RDX within plant foliage. Contrary to these findings, Larson et al. (1999) reported the presence of higher concentrations of high molecular weight explosive transformation product as compared to parent compound in plant tissues. It has also been reported that RDX uptake and distribution is 2 to 16 times more efficient than TNT (Fellows et al. 1995).

Biodegradation of RDX is often attributed to cometabolism in the presence of a primary carbon source under various electron acceptor conditions. RDX can be biodegraded under anaerobic or anoxic conditions by facultative or anaerobic microorganisms (McCormick et al. 1981; Kitts et al. 1994; Freedman and

Sutherland 1998; Hawari et al. 2000; Halasz et al. 2002; Beller 2002). Under aerobic conditions, RDX can be used as a sole source of nitrogen by aerobic microorganisms (Binks et al. 1995; Coleman et al. 1998; Brenner et al. 2000) or by fungus (Bayman et al. 1995; Fernando and Aust 1991; Sheremata and Hawari 2000).

Many laboratory studies have established that anaerobic RDX metabolism occurs more readily than aerobic metabolism, and that hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) are the transient biotransformation intermediates (Figure 1) under anaerobic conditions (Hawari et al. 2000; McCormick et al. 1981; Kitts et al. 1994; Morley et al. 2002; Young et al. 1997; Beller 2002; Freedman and Sutherland 1998; Beller and Tiemeier 2002). In contrast to the ring cleavage metabolites (hydrazine, 1,1-dimethyl- and 1,2-dimethyl-hydrazine, formaldehyde, and methanol) proposed by McCormick et al. (1981), evidence of the formation of methylenedinitramine (MDNA) and bis(hydroxy-methyl)nitramine as a result of ring cleavage has been reported by Hawari et al. (2000). Recent laboratory studies have tentatively identified MDNA as the ring cleavage metabolite during the bioremediation of RDX with anaerobic sludge (Oh et al. 2001; Halasz et al. 2002); however, under in situ conditions it was not detected in any of the samples from an RDX-contaminated aquifer with relatively high levels of MNX, DNX, and TNX (Beller and Tiemeier 2002).

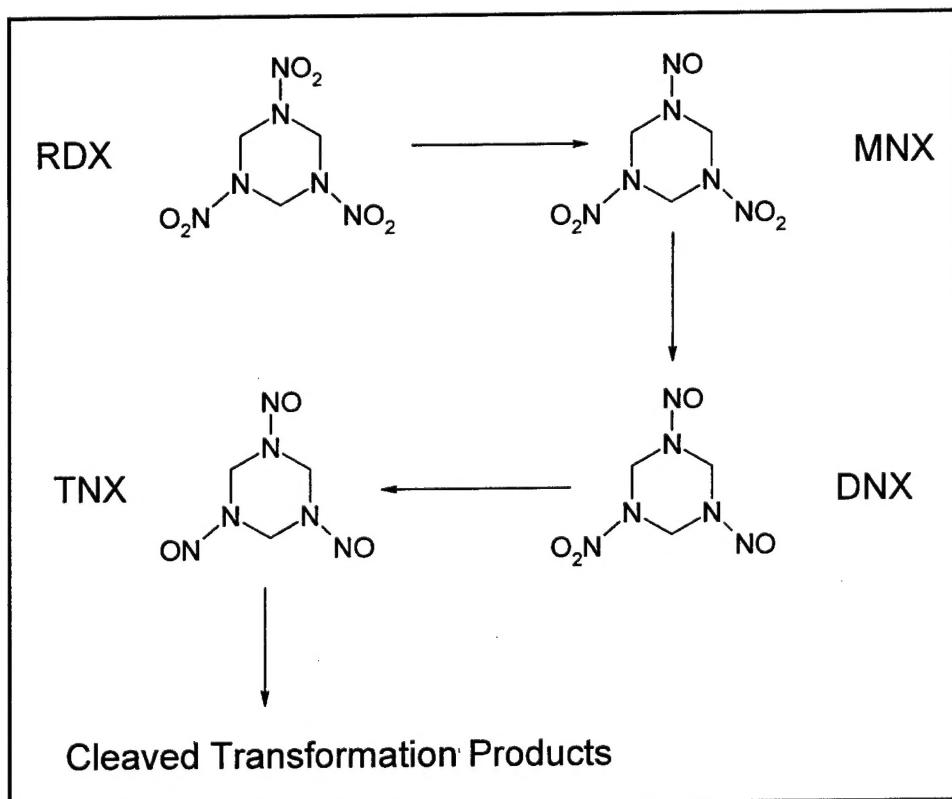


Figure 1. Anaerobic pathway

Although it has been established that RDX can be biodegraded anaerobically by providing a readily available carbon source, electron competition between RDX and other inorganic electron acceptors present in groundwater remains a major factor in achieving the reductive biotransformation of RDX. Co-existence of these ubiquitous electron acceptors in groundwater consumes a major portion of the added electron donor, thus making the process uneconomical and inefficient. Previous studies (Freedman and Sutherland 1998; Beller 2002; Bhushan et al. 2002) have shown that RDX biotransformation is inhibited under nitrate-reducing conditions. The objectives of this research were to explore the phenomenon of electron competition from ubiquitous inorganic electron acceptors (nitrate and sulfate) present in explosives-contaminated groundwater, and to evaluate the effects of these electron acceptors on the rate and extent of RDX biotransformation under simulated in situ condition.

3 Site Description and Sampling

The former NOP is located about 0.8 m (0.5 mile) south of Mead, NE, which is 48 km (30 miles) west of Omaha and 56 km (35 miles) northeast of Lincoln, NE. The NOP covers 69.9 sq km (17,258 acres) in Saunders County. Currently, the land is owned by the University of Nebraska, Agricultural Research and Development Center, U.S. Army National Guard and Reserves, U.S. Department of Commerce, and private interests. The NOP was a load, assemble, and pack facility, which produced bombs, boosters, and shells. Most of the raw materials used to manufacture the weapons at NOP were fabricated at other locations and shipped to NOP for assembly; however, ammonium nitrate was produced onsite for the first months of operation in 1943. The plant was operated intermittently for about 20 years until 1962. During World War II, the production facilities were operated by Nebraska Defense Corporation. Production was terminated for the interim period 1945 through 1949. In 1950, NOP was reactivated to produce an assortment of weapons for use in the Korean conflict. NOP was placed on standby status in 1956 and declared excess to Army needs in 1959.

Detailed information about geological and hydrological characteristics of the NOP site is discussed in Wani et al. (2002). A study conducted by the U.S. Army Corps of Engineers indicated that the explosive contamination in soil at the NOP site is mostly limited to areas in and under drainage ditches and sumps in the load lines and the Bomb Booster area. The soil contamination, often detected as RDX, TNT, and 1,3,5-trinitrobenzene (TNB), is believed to originate from the discharge of water used to wash away explosive dust and residue that resulted from the ordnance load, assemble, and pack process.

Aquifer material at the NOP site was collected from Area 1 (Figure 2) near monitoring well MW-5B from a depth of 11 to 12 m (36 to 40 ft) below ground surface. Soil columns were collected in 5-cm- (2-in.-) diam acetate liners by the direct-push method using a track-mounted mobile sampling device. Further details on aquifer material sampling are presented in the BAZE treatability study report (Wani et al. 2002). The soil columns were thoroughly sealed at both ends to prevent loss of water from the aquifer material during storage and shipping. Samples of aquifer material were transported to the Environmental Laboratory, U.S. Army Engineer Research and Development Center, Vicksburg, MS, via a refrigerated truck.

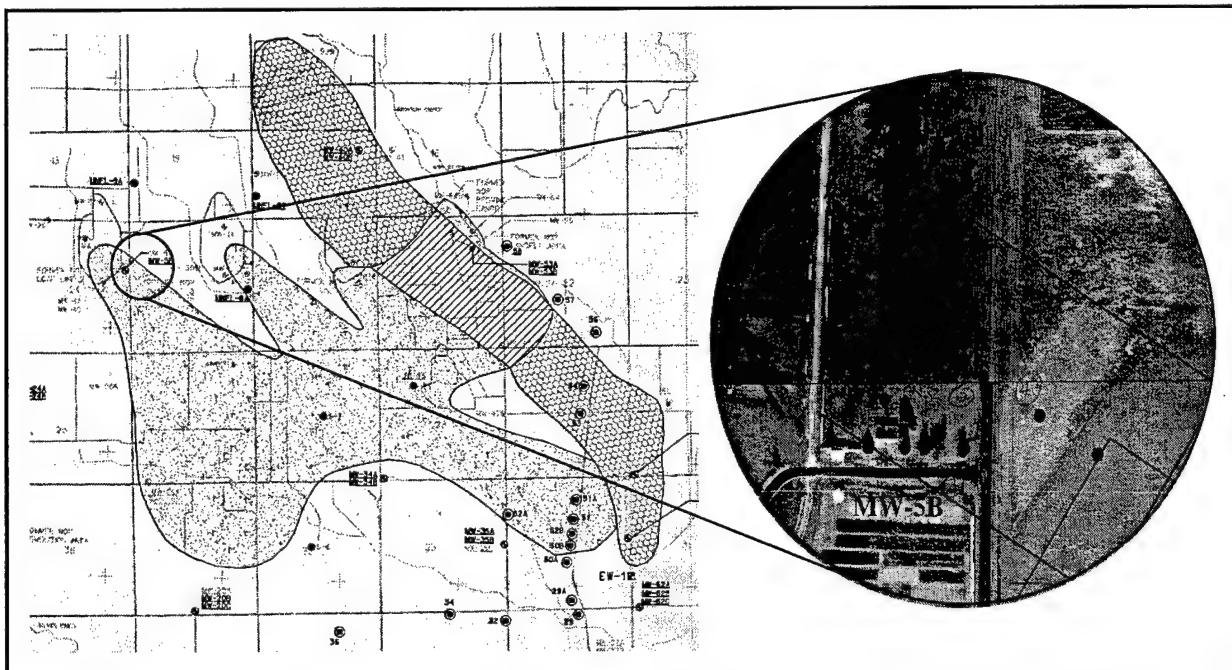


Figure 2. Sampling location at NOP site

4 Materials and Methods

Experimental Setup

Five different column systems (Figure 3) were used to evaluate four different treatments, leaving the remaining one as an experimental control. Each column system was comprised of three 104-cm- (3.4-ft-) long polyvinyl chloride (PVC) columns in series, resulting in an overall column length of 312 cm (10.2 ft), with an inside diameter of 3.8 cm (1.5 in.). The inside diameter of these columns was slightly smaller than that of the acetate liner, which resulted in an increase in total height between the soil cores and the columns. Aquifer material from the acetate liners was slowly emptied into the PVC columns to create minimally disturbed soil cores. Both ends of the columns were closed with PVC caps screened with porous (100 μm) PVC. The columns were connected in series using 3-mm (1/8-in.) stainless steel tubing. Additional sampling ports, 52 cm (1.7 ft) on centers, were placed along the entire length of column system resulting in five intermediate sampling ports in addition to inlet and outlet ports for the development of contaminant bed profiles. These intermediate sampling ports were fitted with 3-mm (1/8-in.) adapters and tubing clamps. Pressure gauges were installed at the inlet to each individual column to examine the effects of microbial growth (biofouling) on water flow, backpressure, and the hydrodynamic properties of the aquifer material. The outlet of each column was equipped with an oxidation-reduction potential (ORP) electrode via a flow-through cell to determine the E_h conditions along the length of the column system. RDX-contaminated water was pumped through the column systems by using variable speed, positive displacement pumps, which allowed the metering of desired water flow through the column systems.

Operation

RDX-contaminated water was prepared by spiking autoclaved, organic-free reagent grade water with RDX stock solution. RDX-contaminated water with a concentration of about $1.16 \pm 0.27 \text{ mg L}^{-1}$ was used in the study. An acetate concentration of 500 mg L^{-1} (as carbon) was used to ensure organic carbon was not the limiting factor. The selection of acetate as the carbon source (electron donor) in this research work stems from other research that suggests that acetate is an excellent electron donor to stimulate in situ microbial reductive conditions (He et al. 2002; Wani et al. 2002).

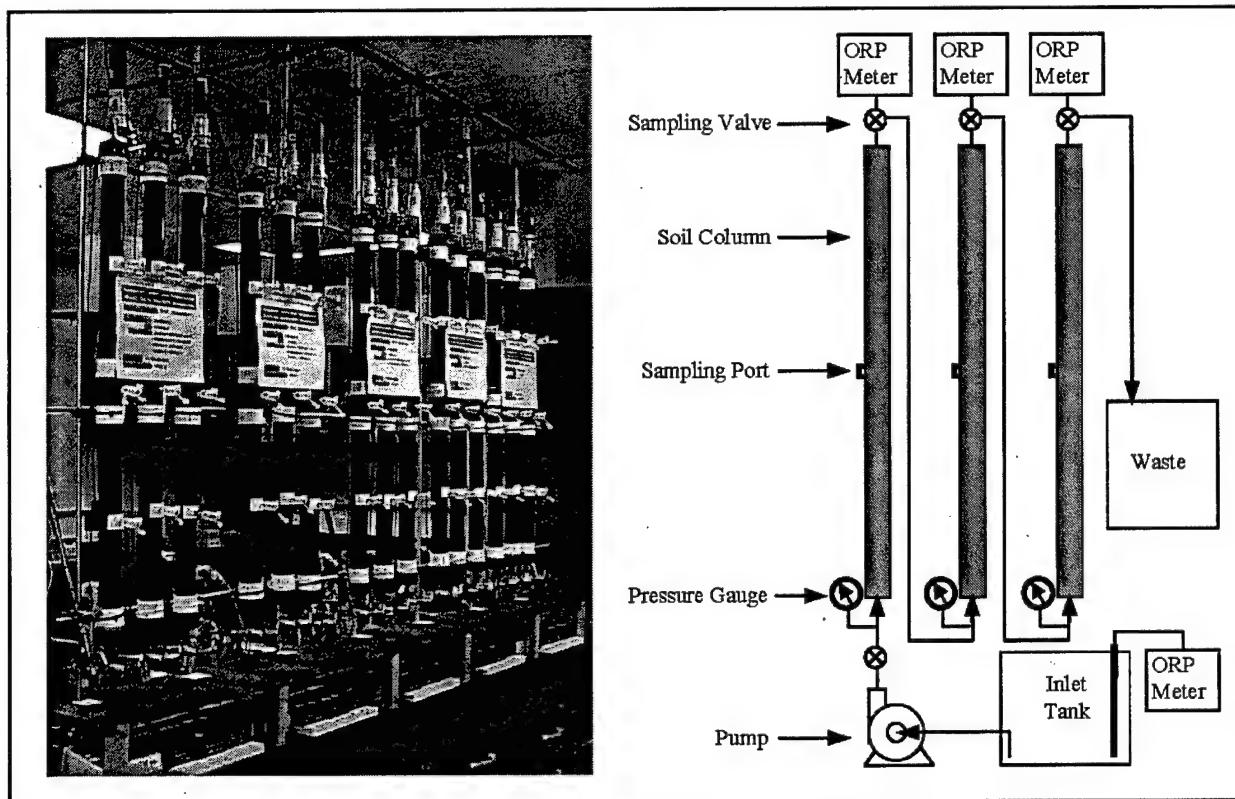


Figure 3. Experimental column setup

In addition to acetate, ammonium was also used to evaluate the synergistic benefits of ammonium on biotransformation of RDX under anaerobic conditions. Two commonly found electron acceptors, nitrate (NO_3^-) and sulfate (SO_4^{2-}), in groundwater were evaluated for their effects on in situ reductive biotransformation of RDX in groundwater. The operating conditions are summarized in Table 1.

Table 1
Column Operating Conditions

Column System	Groundwater Flow mL hr^{-1}	RDX Concentration mg L^{-1}	Amendment Concentration			
			Acetate $\text{mg L}^{-1} \text{C}$	Ammonium mg L^{-1}	Nitrate mg L^{-1}	Sulfate mg L^{-1}
1	10.3 ± 1.1	~ 1.0	~ 500	~ 100	~ 100	~ 100
2	10.4 ± 0.7	~ 1.0	~ 500	~ 100	~ 100	0
3	10.2 ± 0.6	~ 1.0	~ 500	~ 100	0	0
4	10.8 ± 0.5	~ 1.0	~ 500	0	0	0
5	10.5 ± 1.0	~ 1.0	0	0	0	0

Note: Amendment concentrations are nominal.

After completion of the first phase (28 weeks), amendments in Column System #1 were modified to evaluate the impact of high nitrate levels on the rate of RDX biotransformation. Nitrate levels were increased from 100 mg L^{-1} to

500 mg L⁻¹, keeping the other amendment (acetate and sulfate) levels constant. These elevated nitrate levels were continued until Week 36 to attain steady-state conditions, at which time the bed profile analysis was made. For the next 2 weeks sulfate levels were reduced to zero because the results of the previous bed profile showed no sulfate utilization by the biological activity, keeping nitrate and acetate concentrations at 500 mg L⁻¹. Another bed profile with high nitrate and no sulfate was performed on Week 38.

RDX-contaminated water flow through each column was initiated at ~10 mL hr⁻¹ and maintained at this rate throughout the study. Liquid samples were collected from inlet and outlet sampling ports weekly. Samples from intermediate ports along the height of the column were collected every 6 weeks for bed profile analysis. Liquid samples were stored at 4 °C until explosives and amendment analyses.

Analytical Techniques

Acetate, carbonate, chloride, sulfate, nitrate, and nitrite were analyzed on a DIONEX ion chromatograph. Chemical separation and detection were achieved using an Ionpac AS11 analytical column (4 × 250 mm) and a Dionex conductivity detector (1.25-µL internal volume). The gradient analytical method used different concentrations of sodium hydroxide as the mobile phase. For this gradient method the mobile phase flow was 1.5 mL min⁻¹. The sample volume was 25 µL of filtered (0.45 µm) sample. The instrument was calibrated daily from standards prepared from stock solutions. Check standards were run after every 10 samples.

RDX and its nitroso-substituted transformation products analysis was performed using a Waters high-performance liquid chromatograph (HPLC) consisting of a 610 fluid unit pump, a 717 plus autosampler with a 200-µL loop injector, and a Waters 486 tunable ultraviolet absorbance detector. Sample absorbance was monitored at 245 nm. The injection volume was 50 µL. Chemical separation was achieved using a Supelco LC-18 reverse phase HPLC column (25 cm × 4.6 mm (5 µm)) with a Novapak C-18 precolumn for the primary column. The mobile phase comprised of 1:1 (v/v) methanol/organic-free reagent water, flowing at 1.2 mL min⁻¹. For EPA Method 8330 analytes (U.S. EPA 1994), a seven-point calibration curve was utilized. For analysis of breakdown products of the 8330 analytes, a five-point calibration curve was used. The HPLC was calibrated daily from standards prepared from stock solutions. Check standards were run after every 10 samples.

ORP and pH were measured with electrodes that were calibrated weekly. Both ORP and pH were measured with Oakton WD-35100-00 model pH/ORP controllers (Cole-Parmer, Vernon Hills, IL) with a measuring range of 0 to 14 for pH and -1250 to 1250 mV ORP. ORP was measured using a Cole-Parmer combination redox electrode with platinum sensing surface and Ag/AgCl reference electrode. The value of E_h was obtained by adding standard potential of the reference electrode (E_R) to the measured potential (E). For this ORP electrode, E_R at 25 °C (room temperature) was 202 mV. At 25 °C, the ORPs of

quinhydrone-saturated solutions in pH 4 and pH 7 buffer solutions were within the designed ranges of 263 and 86 mV, respectively. The pH was determined with a Cole-Parmer combination electrode.

Column Hydrodynamics and Biotransformation Kinetics

A tracer test was performed to evaluate the hydrodynamic properties of the aquifer material in the column system. Tracer tests were performed by adding chloride at nontoxic levels and sampling the effluent periodically to develop a breakthrough curve. An advection-dispersion model was fitted to the data to determine dispersivity and bed porosity.

$$\frac{\partial C}{\partial t} = \alpha v \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} \quad (1)$$

where

C = chloride concentration, M L⁻³

t = time elapsed, t

α = dispersivity, L

$v = q/(A*n)$ = average interstitial or pore water velocity, L t⁻¹

q = water flow, L³ t⁻¹

A = column cross-section area, L²

n = aquifer material porosity

x = distance from column inlet, L

Given the initial condition $C(x,0) = C_i$, and boundary conditions $C(0,t) = C_0$ and $\partial C/\partial x(\infty,t) = 0$, the solution to Equation 1 is shown in Equation 2:

$$C = C_i + \left(\frac{C_o - C_i}{2} \right) \left[erfc \left(\frac{x-vt}{2\sqrt{\alpha vt}} \right) + \exp \left(\frac{x}{\alpha} \right) erfc \left(\frac{x+vt}{2\sqrt{\alpha vt}} \right) \right] \quad (2)$$

The rate of RDX biotransformation was determined by sampling at the intermediate ports in the column system. A contaminant profile was developed and an advection-dispersion model (Equation 3) for contaminant transport with decay was fitted to the results. The bed-profile sampling was done every 6 weeks when the operating conditions were steady and columns had reached equilibrium conditions with steady RDX removal.

$$\frac{\partial C}{\partial t} = \alpha v \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} - kC \quad (3)$$

where k = RDX first-order biodegradation rate coefficient, t^{-1} .

With boundary conditions $C(0,t) = C_0$ and $\partial C / \partial t(\infty, t) = 0$, Equation 3 can be solved to Equation 4 as follows:

$$C = C_0 \cdot \exp \left[\left(\frac{x}{2\alpha v} \right) \left(v - \sqrt{v^2 + 4k\alpha v} \right) \right] \quad (4)$$

5 Results

Column Hydrodynamics

The results of tracer tests are shown in Figure 4. The tracer test results revealed that the aquifer material tested has an effective porosity of 0.31 ± 0.02 and a dispersivity range of 1.81 to 5.03 cm (0.72 to 2.01 in.). The measured porosities are well within the values expected for a sandy soil. However, the dispersivities are smaller than would be expected in the field but are typical for laboratory evaluations.

Plots of operating conditions (volumetric flow, changes in liquid stream E_h and pH as it flowed through the soil columns, and changes in backpressure) for individual column systems are shown in Figures 5-7. RDX-contaminated water flow throughout the study was relatively constant at $10.47 \pm 0.41 \text{ mL hr}^{-1}$ in all five column systems and fluctuated slightly from the designed flow (10 mL hr^{-1}). This average flow of RDX-contaminated water in the column systems resulted in a velocity of about 0.72 m d^{-1} (2.34 ft d^{-1}), which is comparable with the NOP site groundwater velocity of approximately 0.61 m d^{-1} (2 ft d^{-1}). Influent pH varied between 6.5 and 7.5 in treatment column systems where RDX-contaminated water was amended with acetate, ammonium, nitrate, and sulfate. In the control column system, influent pH was slightly higher (8 to 9). Effluent from all four treatment column systems showed a slight increase in pH (8 to 8.6); however, in the control column system there was no measurable change in the pH (Figure 5).

Reducing conditions were established in each column system as shown in Figure 6. In treatment column systems E_h dropped from about 450 mV to around -350 mV. Anaerobic conditions were established in soil column systems by providing a carbon source to the indigenous microorganisms, which then quickly depleted available oxygen in the feed water to create a reduced environment. The drop in redox potential in the control column system was similar to the treatment column systems; however, later in the study the redox levels in the effluent were not as low, especially at the outlet from the first column (Figure 6). This was mainly because the soil (aquifer material) used in these column systems was initially used for the BAZE treatability study (Wani et al. 2002) using site-specific groundwater, which contained significant quantities of organic carbon, iron, and other groundwater chemical components.

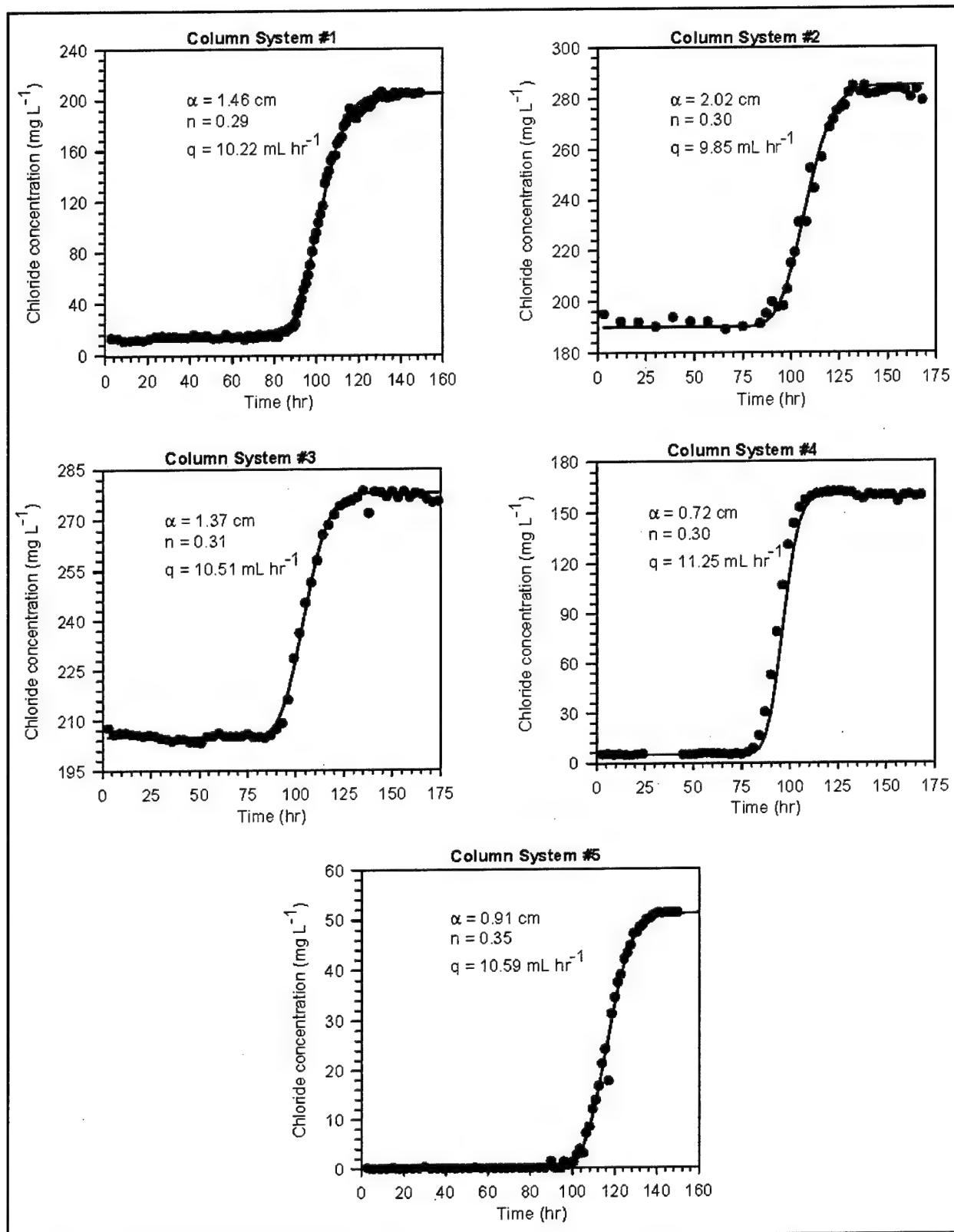


Figure 4. Chloride tracer breakthrough curves for individual column systems (α = dispersivity, n = aquifer material porosity, and q = water flow)

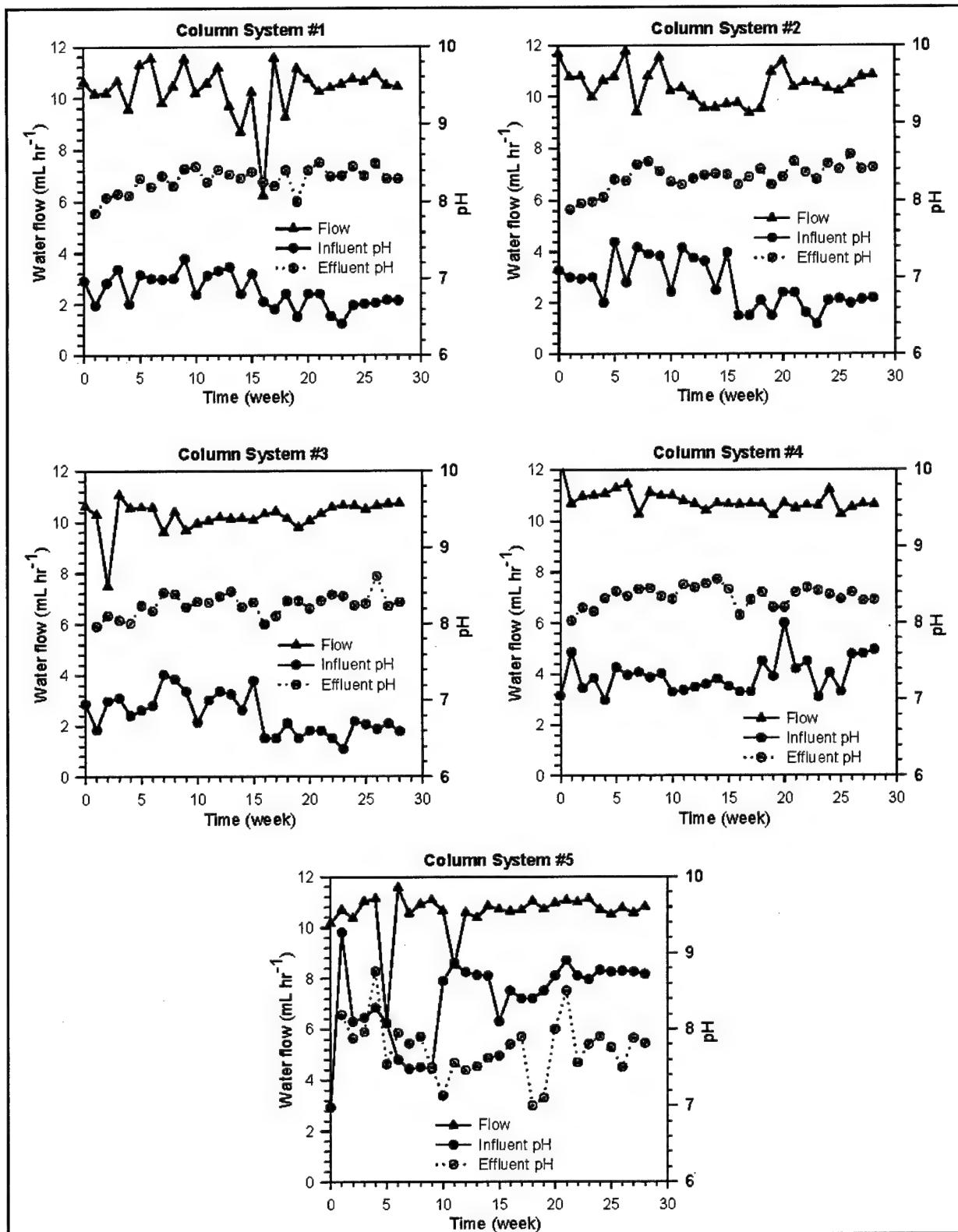


Figure 5. RDX-contaminated water flow and pH in individual column system

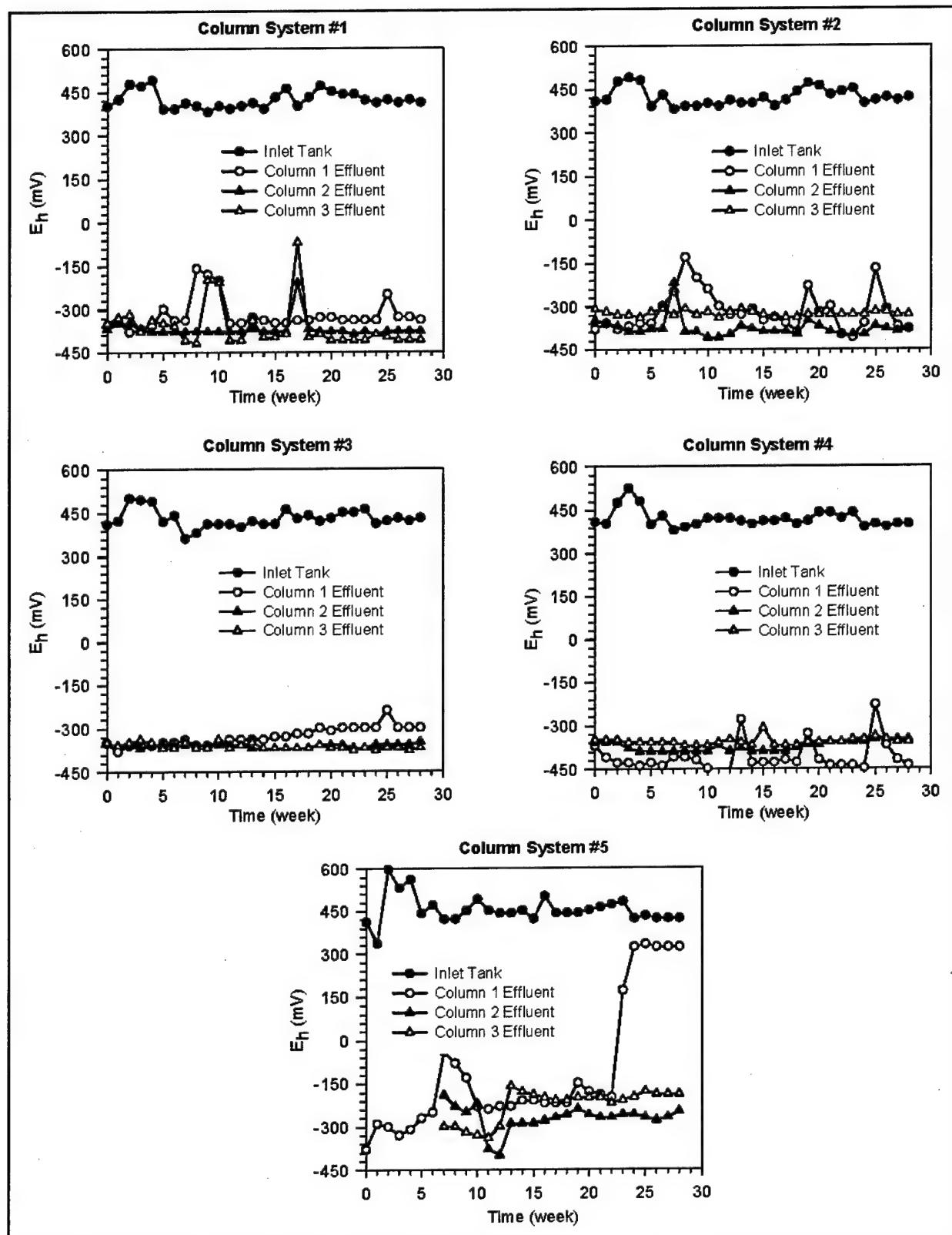


Figure 6. Redox potential at column inlets and outlets in each column system

There was no significant backpressure buildup due to biofouling in any of the column systems during the entire 28-week study (Figure 7). Occasional hikes in the backpressure were mainly due to plugging of the porous PVC screen at the column inlets due to extracellular secretions from biomass. After cleaning or replacing the porous PVC screens, this flow resistance was removed, and pressure loss across the columns dropped to initial levels.

RDX Biotransformation

RDX concentrations in the influent stream, ranging between 1 and 1.8 mg L⁻¹, were reduced to below detection levels of 20 µg L⁻¹ in each treatment column system. Traces of nitroso-substituted transformation products (MNX, DNX, and TNX) were occasionally observed in the effluent from the amendment-treatment column systems (Figure 8). Significant quantities of nitroso-substituted transformation products were observed in the effluent from the amendment-control column system. Although no carbon source was used in the control column system, organic carbon present in the aquifer material was used by the biological activity in creating reduced conditions. Additionally this column system was used in the previous BAZE treatability study (Wani et al. 2002) using site groundwater with elevated levels of organic carbon and other reducing substances such as sulfide and ferrous iron, some of which might have sorbed on the aquifer material. Initially for the first 11 weeks, only TNX was observed in the amendment-control column effluent. However, later on as the organic carbon present in the aquifer material was used, effluent E_h increased (Figure 6) and measurable quantities of MNX, DNX, and TNX were identified in the effluent.

The formation and identification of different transformation products in amendment-treatment and amendment-control column systems identify the need for a low redox environment for complete transformation of RDX and subsequent nitroso-substituted intermediates. In treatment column systems where redox potential was very low (approximately -350 mV), RDX was transformed into nonnitroso metabolites other than MNX, DNX, and TNX. These nonnitroso metabolites may include the nonvolatile transformation products like hydrazines, MDNX, formaldehyde, methanol, etc., proposed by other researchers (McCormick et al. 1981; Hawari et al. 2000), in addition to mineralization CO₂. In the amendment-control column system where redox potential was positive (300 mV) in the first column segment and negative (-150 mV) in next two column segments, significant levels of MNX, DNX, and TNX were observed in the effluent (Figure 8). Under in situ conditions similar results were reported by Beller and Tiemeier (2002) that in the wells with the detectable MNX, DNX, and TNX levels, E_h (10 to 170 mV (median 80 mV)) did not indicate strongly reducing conditions. This type of sequential reductive biotransformation of RDX has been reported for various RDX-metabolizing cultures that used organic electron donors (McCormick et al. 1981; Freedman and Sutherland 1998; Hawari et al. 2000; Beller 2002).

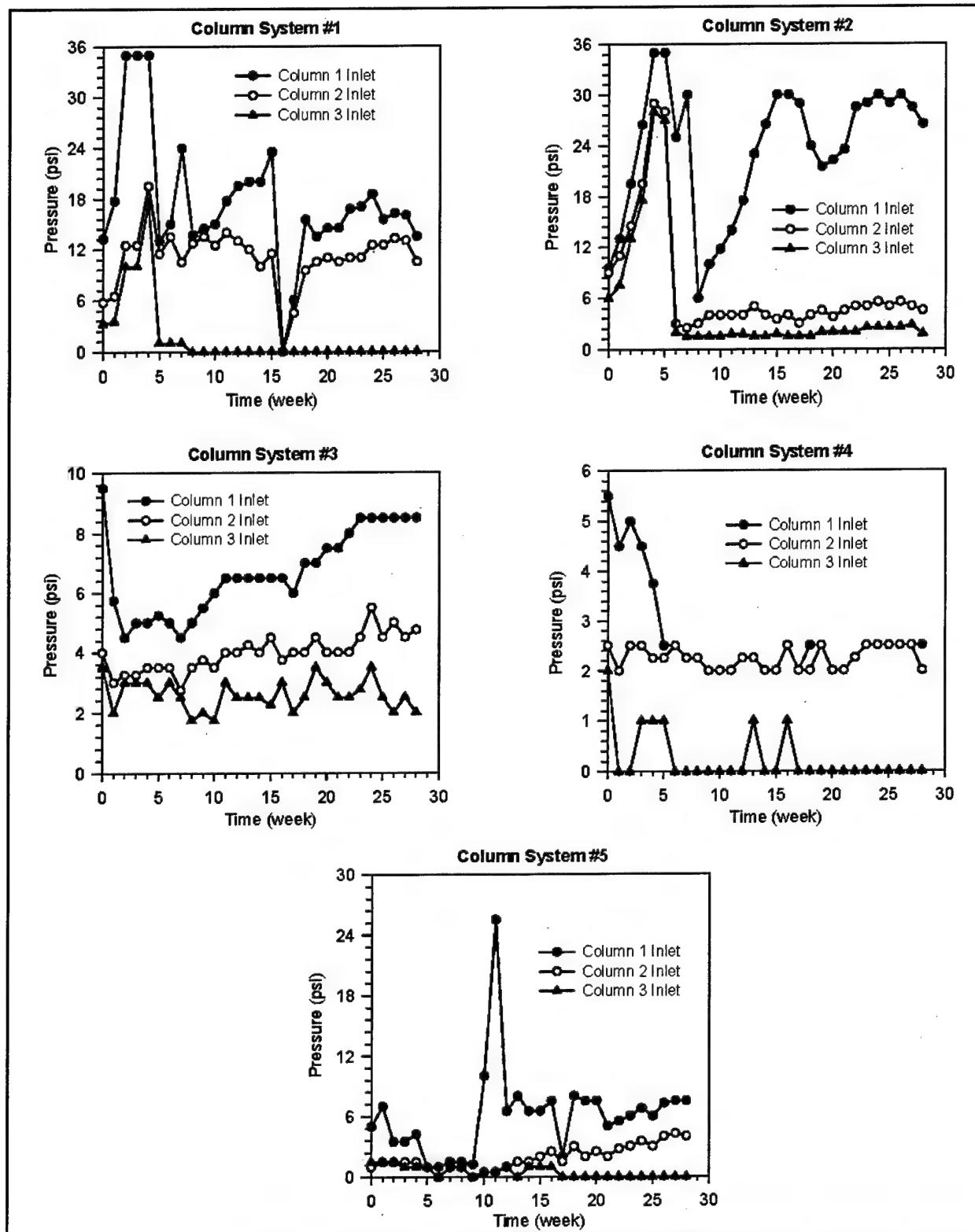


Figure 7. Flow resistance and backpressure in individual column system (pressure at Column 1 inlet is a cumulative pressure of all the three columns, and at Column 2 inlet is the sum of backpressure in Columns 2 and 3. Note: to convert psi to kPa multiply by 6.9)

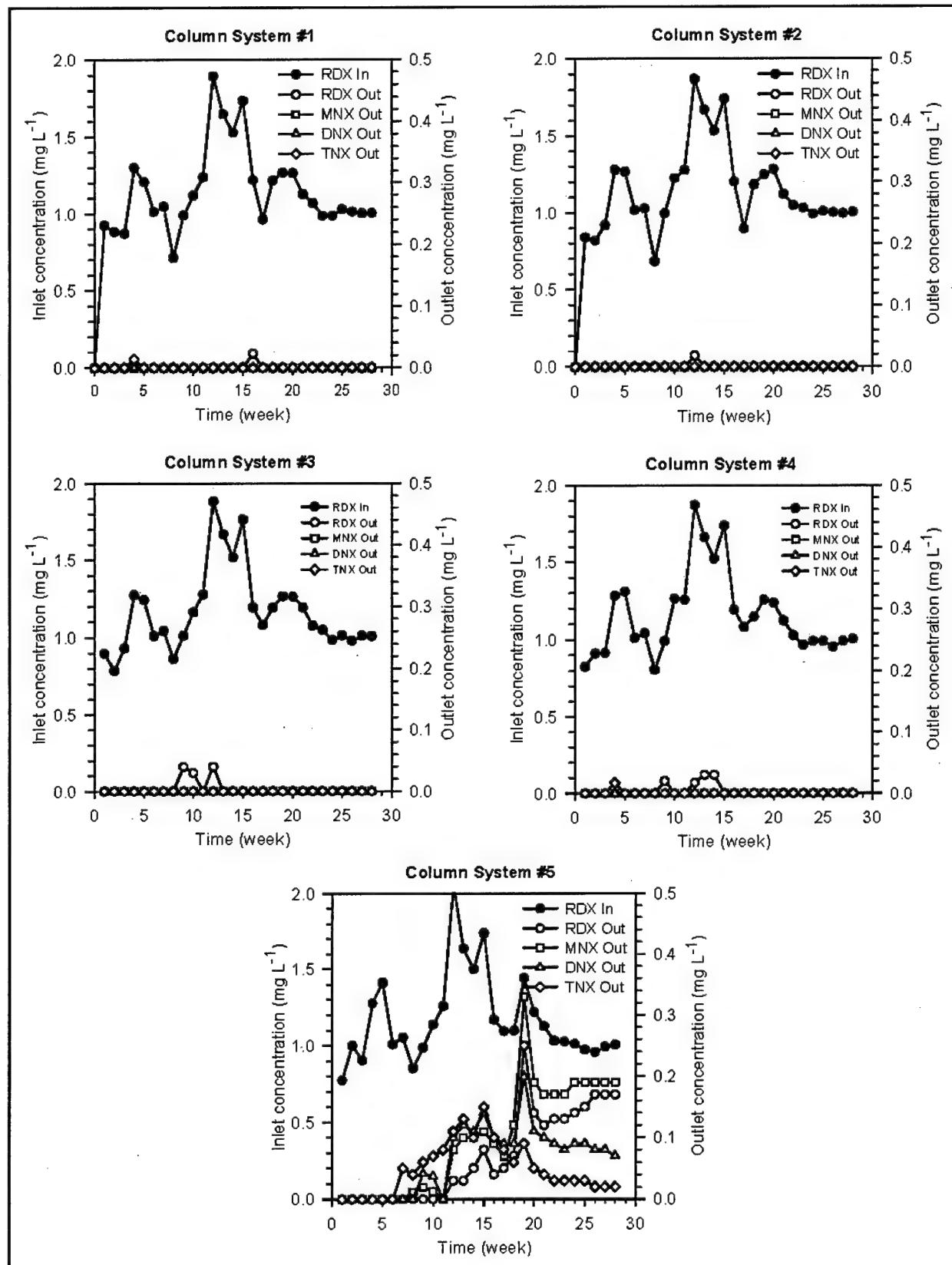


Figure 8. RDX and nitroso-RDX intermediates concentration in influent and effluent stream

In the amendment-control column system, during the last 8 weeks, on an average, the cumulative concentration of these nitroso-substituted transformation products in the effluent accounted for about 30 percent of the inlet RDX concentration on a molar basis. With unused RDX in the effluent stream, the total molar concentration of RDX, MNX, DNX, and TNX was approximately 45 to 50 percent of the inlet RDX concentration (Table 2), leaving about 50 percent of the inlet RDX unaccounted for in terms of nitroso-substituted RDX intermediates, which might include mineralization CO_2 and other nonnitroso-transformation products.

Table 2
Proportions of RDX, MNX, DNX, and TNX in Control Column System

RDX and Nitroso Derivatives	Week #20		Week #25		Week #28	
	mg L ⁻¹	μM	mg L ⁻¹	μM	mg L ⁻¹	μM
Influent RDX	1.220	5.50	0.973	4.38	1.004	4.52
Effluent RDX	0.137	0.62	0.150	0.68	0.172	0.77
Effluent MNX	0.187	0.91	0.186	0.90	0.185	0.90
Effluent DNX	0.110	0.58	0.085	0.45	0.072	0.38
Effluent TNX	0.050	0.29	0.027	0.16	0.023	0.13
Total Effluent		2.39		2.18		2.18

It is hypothesized that two redox-dependent degradation pathways may be present. One pathway is the sequential reduction of nitro functional groups to nitroso functionality. The degradation rates may be faster than the resolution accorded in this column study. A second pathway may be direct attack on the ring. This direct attack, resulting in ring cleavage, may be active only at low redox potentials (McCormick et al. 1981; Hawari et al. 2000). Similar results of nonnitroso-substituted biotransformation of [¹⁴C]RDX by aquifer microorganisms under anaerobic conditions were reported by Beller (2002), in which he observed that the nonvolatile metabolites MNX, DNX, and TNX constituted ≤ 0.5 percent of the total RDX carbon. In another study, Oh et al. (2001) reported that although MNX, DNX, and TNX were detected in microcosms amended with zero valent iron and anaerobic sludge, these nitroso-substituted RDX intermediates never accumulated above 5 percent of the added RDX. These researchers tentatively identified a soluble intermediate MDNA that resulted from ring cleavage. However, under natural in situ conditions MDNA was not detected in any of the samples from an RDX-contaminated groundwater aquifer, and MNX was typically the most abundant of the three nitroso-substituted transformation products (Beller and Tiemeier 2002).

In all treatment column systems, except the column system amended with acetate plus ammonium, the influent acetate concentrations (500 mg L⁻¹, as carbon) were partially used by the biological activity in the aquifer material. In the acetate-plus-ammonium-amended column system almost all of the acetate in the influent stream was used by the biological activity (Figure 9). One plausible reason for the complete utilization of influent acetate in this column system may be increased biological activity by resident microorganisms because of the availability of ammonium, a more favorable nitrogen source. Nonetheless, the

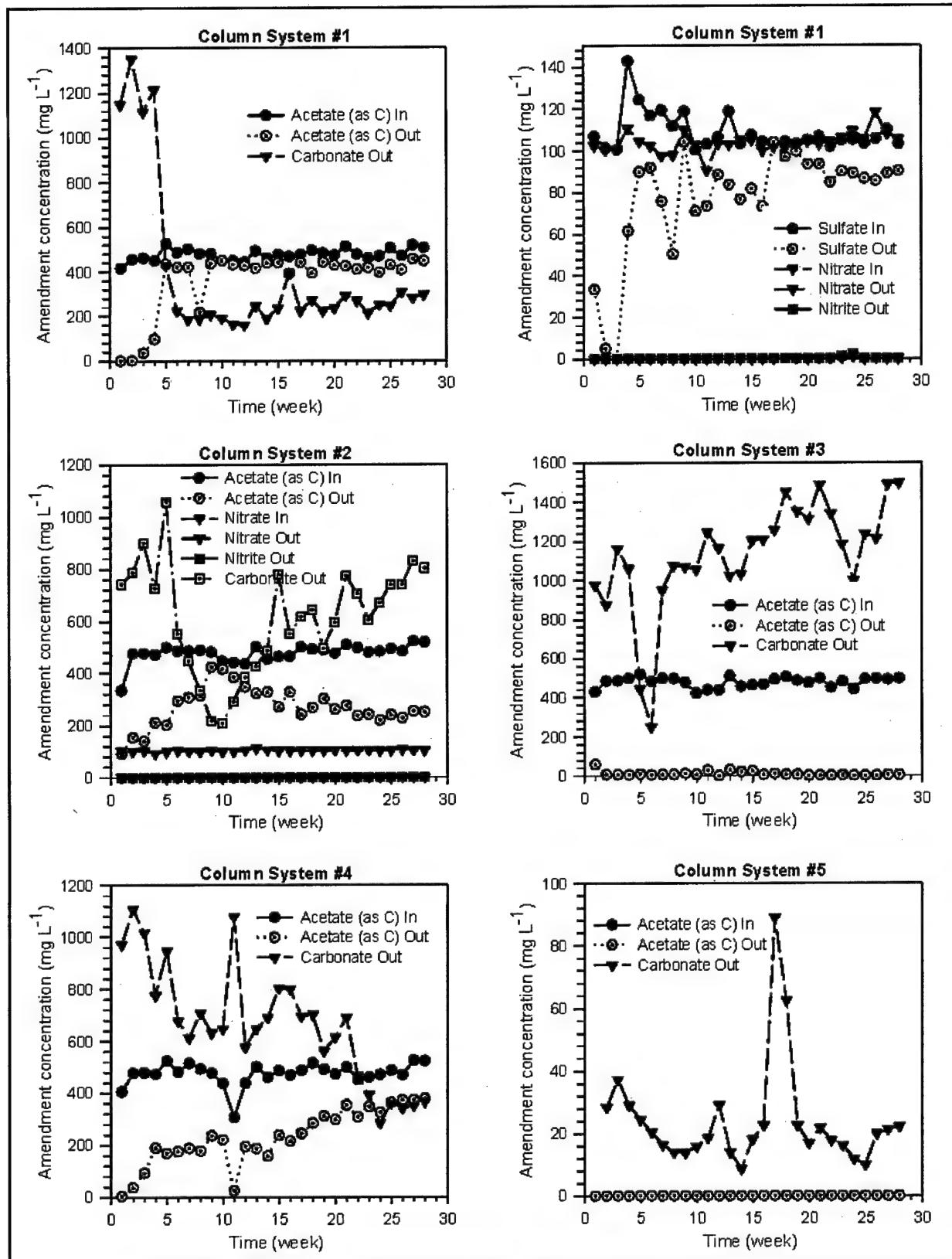


Figure 9. Amendment concentration in column influent and effluent streams

presence of ammonium did not have any positive influence on RDX biotransformation. These findings are similar to those reported by Beller (2002) that ammonium had no beneficial effects on RDX degradation.

Influent nitrate levels (100 mg L^{-1}) in column systems amended with nitrate in addition to carbon source were completely denitrified, without any detection of nitrate or nitrite in the effluent (Figure 9). These nitrate levels did not halt the RDX biodegradation, probably because the nitrate levels were low as compared to concentration of readily biodegradable electron donor (acetate) that exceeded the amount needed for nitrate reduction. Influent sulfate (100 mg L^{-1}) in the sulfate-amended column system was initially used by the biological activity; however, after the first 5 weeks more than 90 percent of the influent sulfate was unused (Figure 9). This lack of sulfate reduction may be the result of (a) the absence of sulfate-reducing bacteria, or (b) inhibition from RDX transformation products.

RDX Biotransformation Kinetics

The RDX transformation rate in individual column systems was evaluated using the RDX concentration profile along the column length. Three bed profile samplings were carried out to determine the average rate of RDX biotransformation with time of operation. Bed profile samples were collected from intermediate sampling ports along the column length at Weeks 12, 18, and 24 when the columns were operating at equilibrium with steady RDX removal (Figures 10-12). In all bed profile analyses, axial explosives concentration profiles were the same for the individual column system; however, the presence of different amendment mixture(s) resulted in the formation of different nitroso-substituted metabolites at intermediate sampling points. For example, in all three bed profile analyses, TNX was the only transformation product detected in the column system amended with acetate alone, whereas in the remaining three treatment column systems, the predominant nitroso-substituted transformation product was MNX. This pattern of transformation products may be a result of the presence of different microbial consortia because all treatment column systems where MNX was the predominant metabolite had ammonium in the amendment mixture, which might have changed the microbial dynamics. Kitts et al. (1994) observed similar variable microbial ability to transform RDX. The researchers reported that two species (*Morganella morganii* and *Providencia rettgeri*) completely transformed RDX and subsequent nitroso-substituted intermediates, and the third one (*Citrobacter freundii*) partially transformed RDX and generated high concentrations of nitroso-substituted intermediates.

Initial MNX increases in Column Systems #1, #2, and #3 corresponded to the rapid RDX losses, suggesting that a likely first step in the RDX biodegradation pathway by the resident microorganisms under these experimental conditions is the formation of MNX by reduction of a single RDX nitro ($-\text{NO}_2$) group to nitroso ($-\text{NO}$) group. MNX has been reported to be the most abundant of the three nitroso-derivates of RDX under anaerobic conditions (Beller and Tiemeier 2002; Bhushan et al. 2002). In these treatment column systems, MNX concentration peaked at 52 cm (1.7 ft) from the column inlet and then gradually decreased, presumably by further conversion to DNX, TNX, or other degradates.

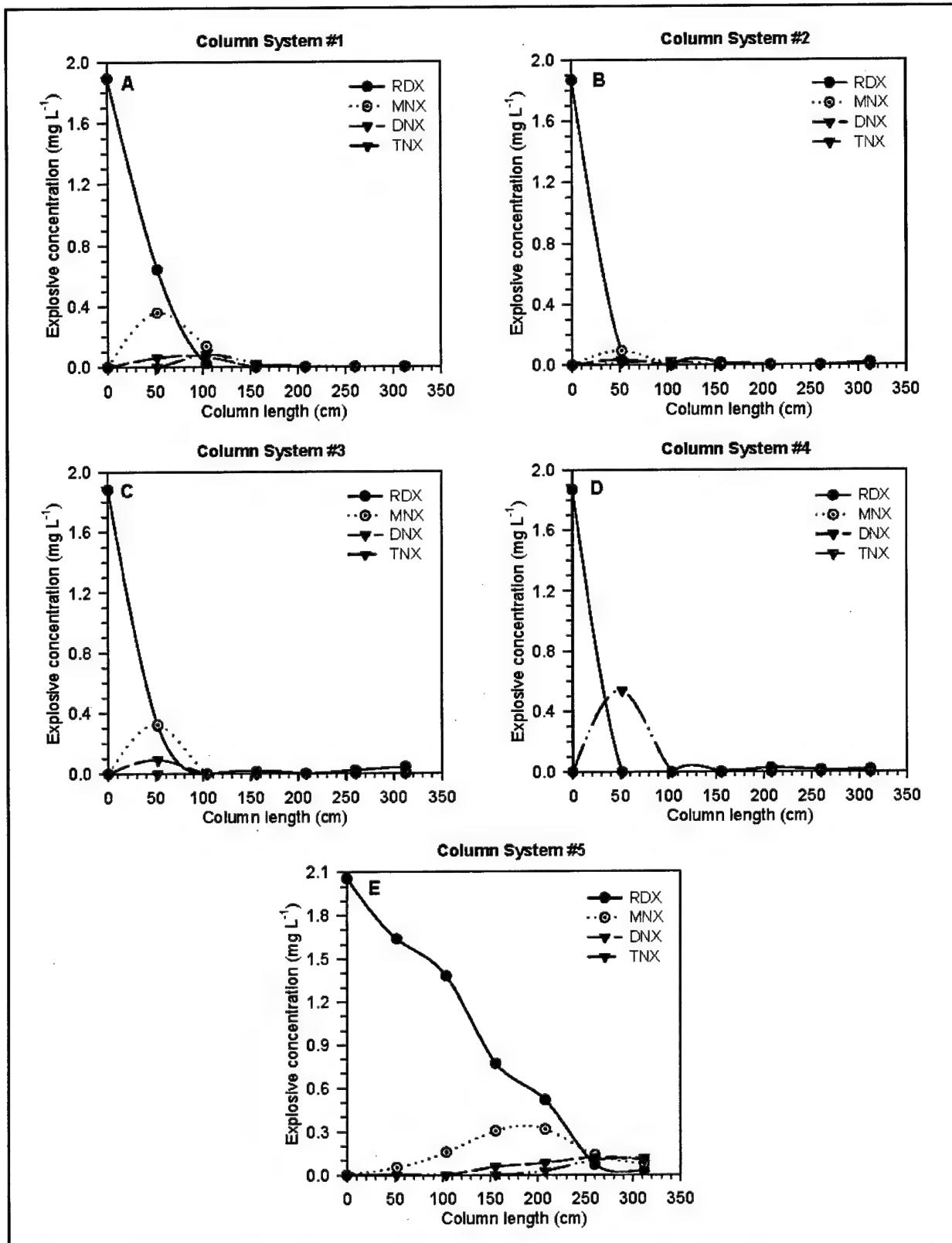


Figure 10. Axial concentration profile of RDX and its transformation products at Week 12 (Bed Profile #1)

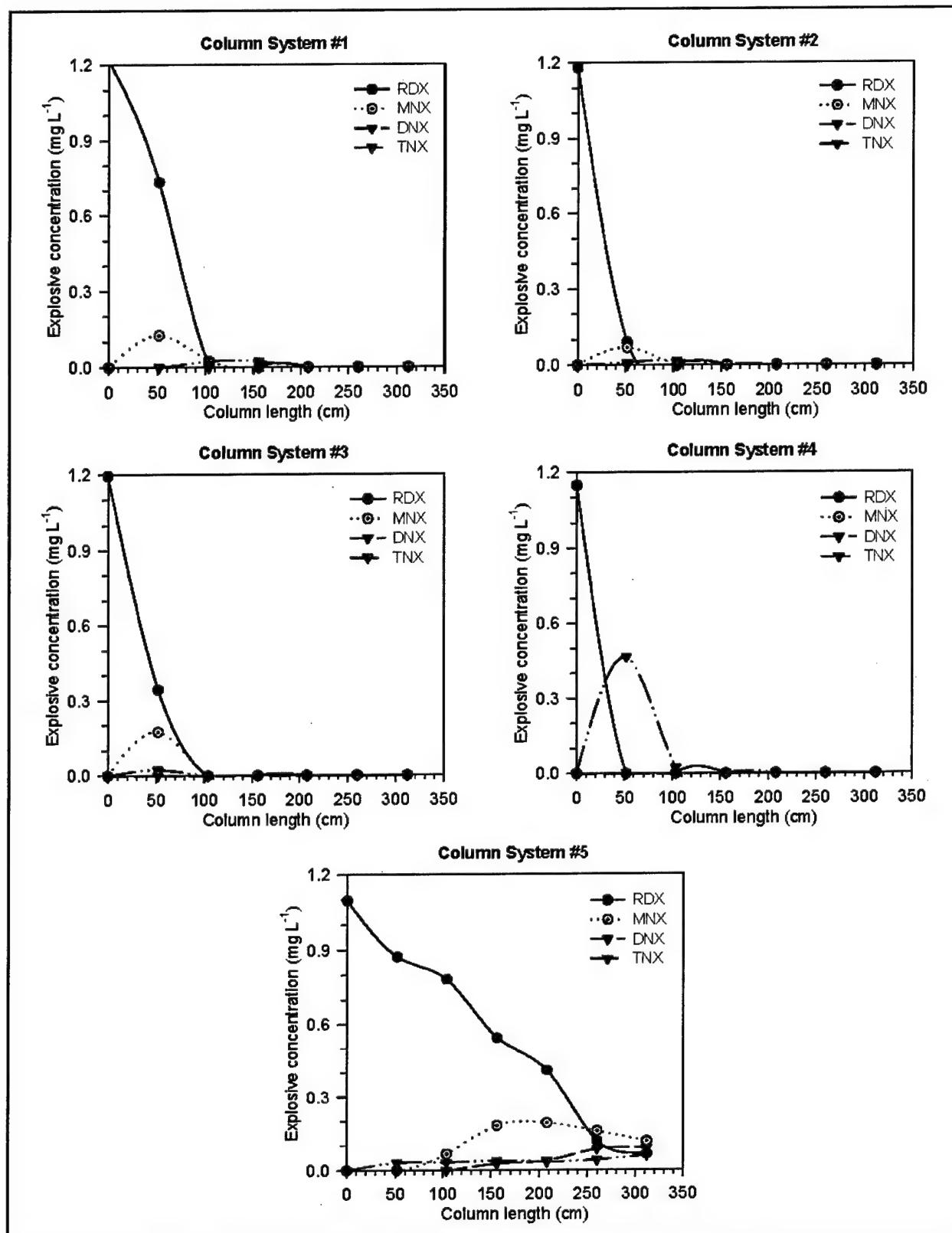


Figure 11. Axial concentration profile of RDX and its transformation products at Week 18 (Bed Profile #2)

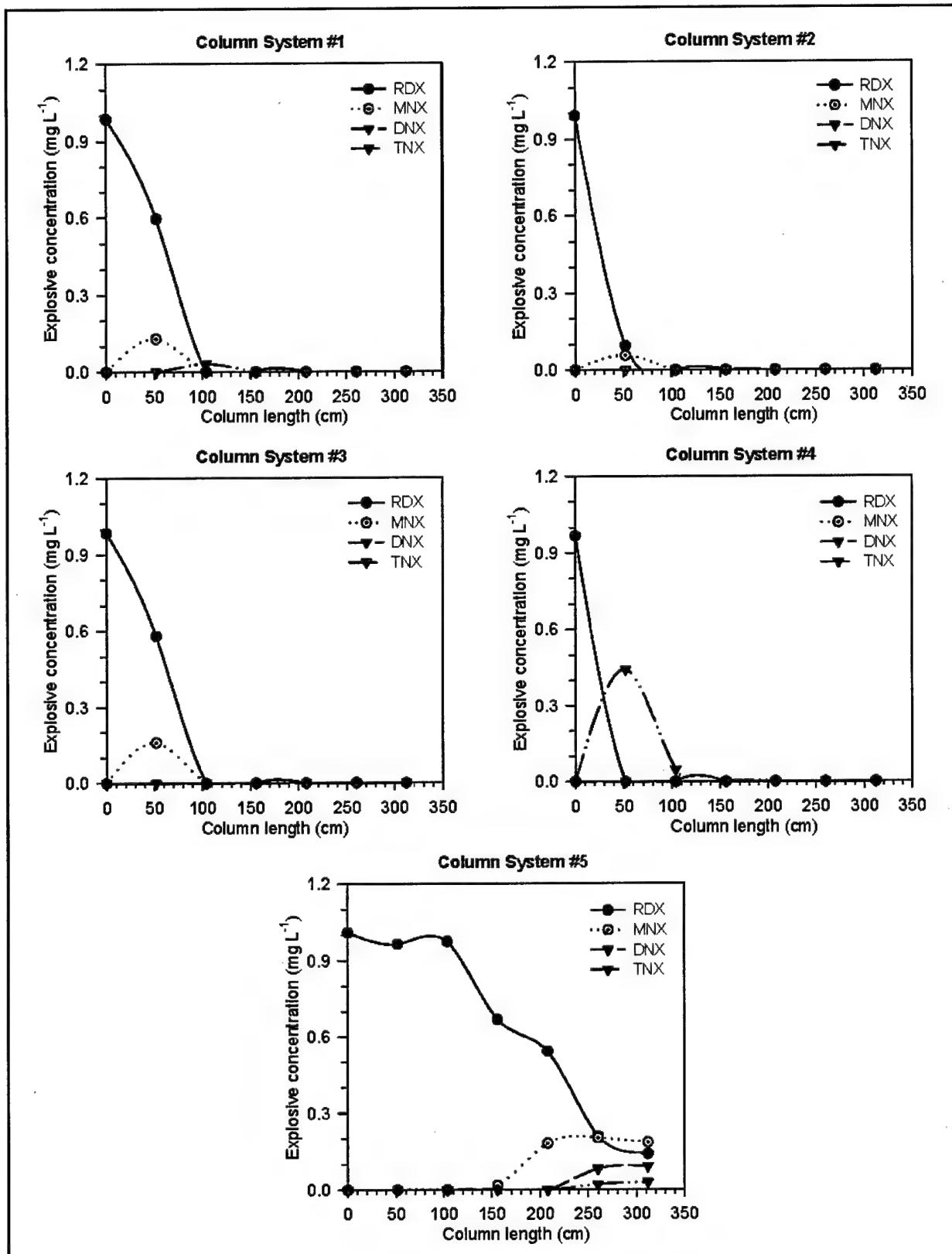


Figure 12. Axial concentration profile of RDX and its transformation products at Week 24 (Bed Profile #3)

In the amendment-control column system all three nitroso-substituted transformation products were observed along the column length. MNX concentration increased at the beginning of the column system corresponding to the rapid decrease in RDX concentration. Along the column system length, MNX concentration gradually decreased with an increase in DNX and TNX concentrations. These transformation products, along with measurable quantities of RDX, were observed all along the column length and in the effluent (Figures 10-12). The predominant transformation product in the amendment-control column system was MNX, and TNX was observed at relatively low concentrations. With the time of operation, as the organic carbon present in aquifer material was used by the biological activity, the redox potential increased and no RDX biotransformation was observed. For example, in the last bed profile (Figure 12) there was no biotransformation of RDX in the first 104 cm (3.4 ft) because the redox potential at the outlet of the first column segment (i.e., first 104 cm) was positive (>300 mV) during the last 8 weeks (Figure 6). However, within the next 208 cm (6.8 ft) of column length, where E_h was between -200 and -300 mV, RDX was rapidly biotransformed into nitroso-substituted intermediates because of reduced conditions.

The axial concentration profile of amendments in the column systems is shown in Figures 13-15. In the treatment column system where sulfate was in the amendment mixture, the influent sulfate concentration of ~ 100 mg L⁻¹ was essentially unused in the biological activity throughout the study, probably because of the lack of sulfate-reducing bacteria or inhibition from RDX transformation products. This sulfate concentration slightly delayed the RDX removal along the column length. In the treatment column systems with nitrate in the amendment mixtures, influent nitrate levels (~ 100 mg L⁻¹) were completely removed by the biological activity within the first 52 cm (1.7 ft) of the column system. Influent acetate concentrations of about 500 mg L⁻¹ were partially used by the biologically activity in all amendment-treatment columns except in the treatment column with acetate and ammonium as the amendments. In all the treatment column systems significant amounts of carbonate were observed in the effluent as well as at intermediate ports along the column length. Carbonate concentration increased as the acetate concentration declined along the column length. Low levels of carbonate were also identified at intermediate ports in the amendment-control column, although no acetate was used in this column.

The rate of RDX biotransformation in the presence of different amendment mixture(s) was evaluated by fitting the steady-state advection-dispersion model with contaminant decay (Equation 4) to the axial RDX concentration profiles obtained in the three bed profile tests described above. The plots of these kinetic analysis are illustrated in Figures 16-18. The average RDX biodegradation kinetic parameters estimated from the three bed profile analyses for the individual column systems are summarized in Table 3.

The first-order biodegradation rate coefficient for RDX was highest (0.79 hr⁻¹) in the treatment column system amended with acetate only. The rate coefficient is about 52 times higher than the rate coefficient (0.015 hr⁻¹) for RDX in the amendment-control column system. The presence of electron acceptors (nitrate and sulfate) significantly (95-percent confidence) reduced the RDX

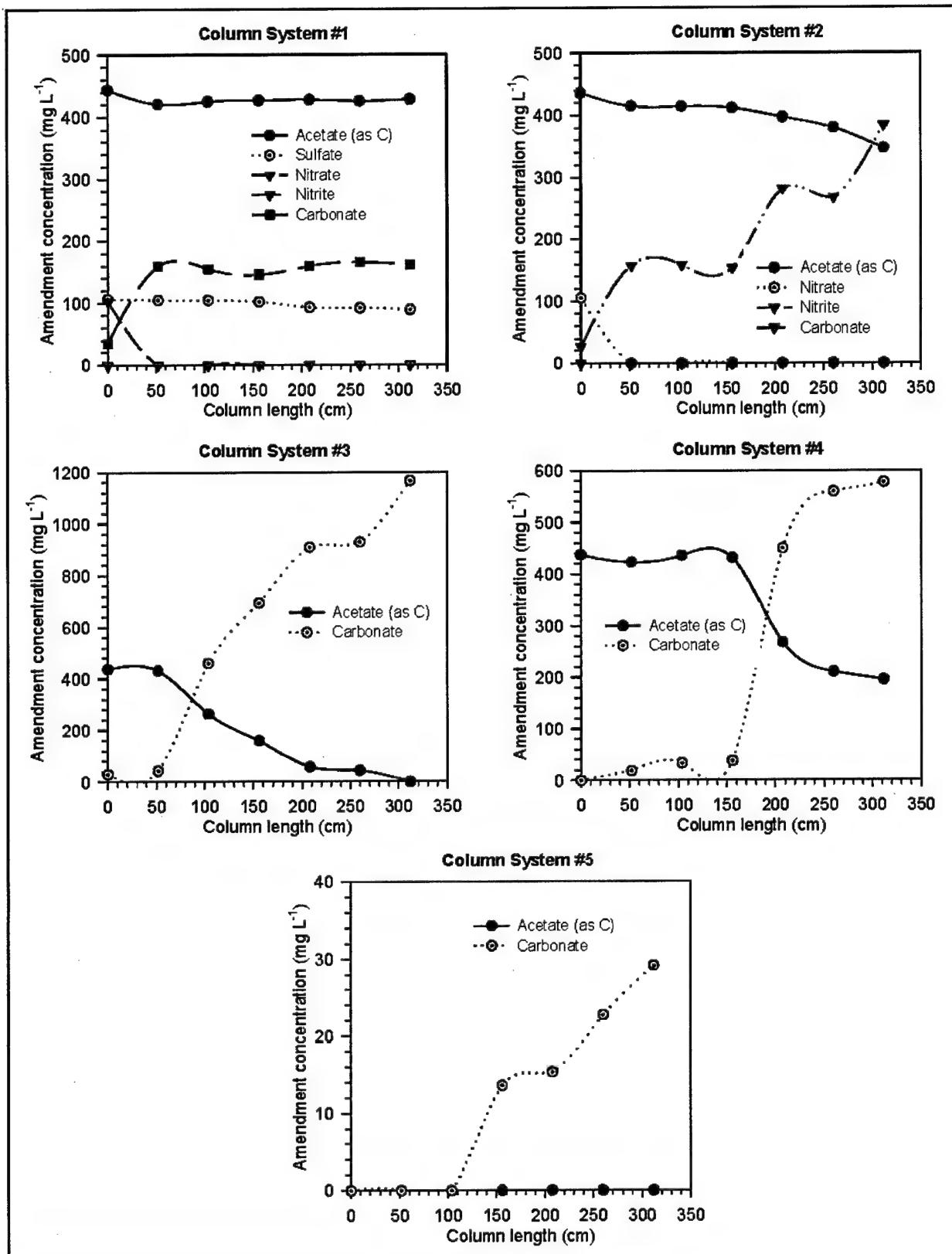


Figure 13. Axial concentration profile of amendments at Week 12 (Bed Profile #1)

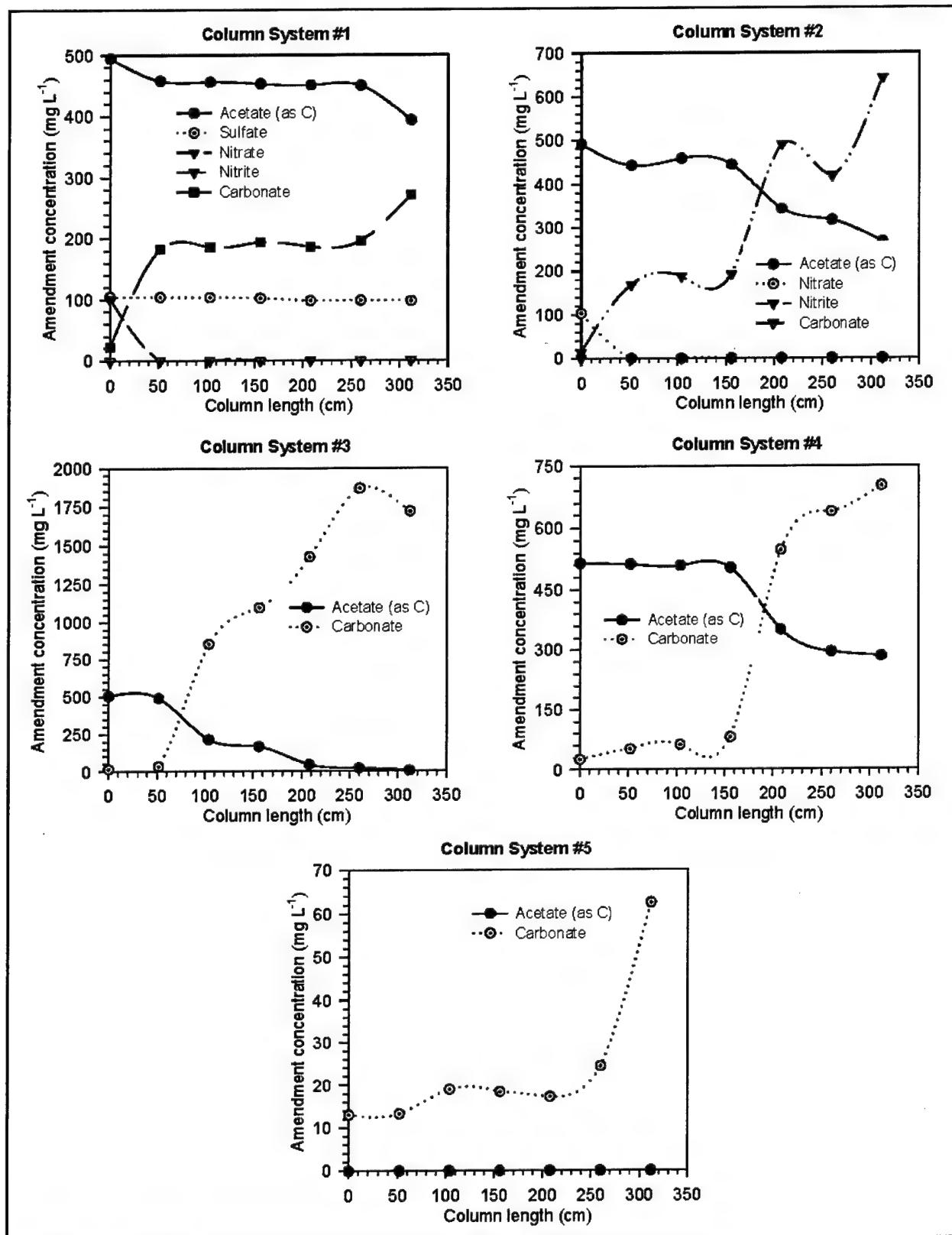


Figure 14. Axial concentration profile of amendments at Week 18 (Bed Profile #2)

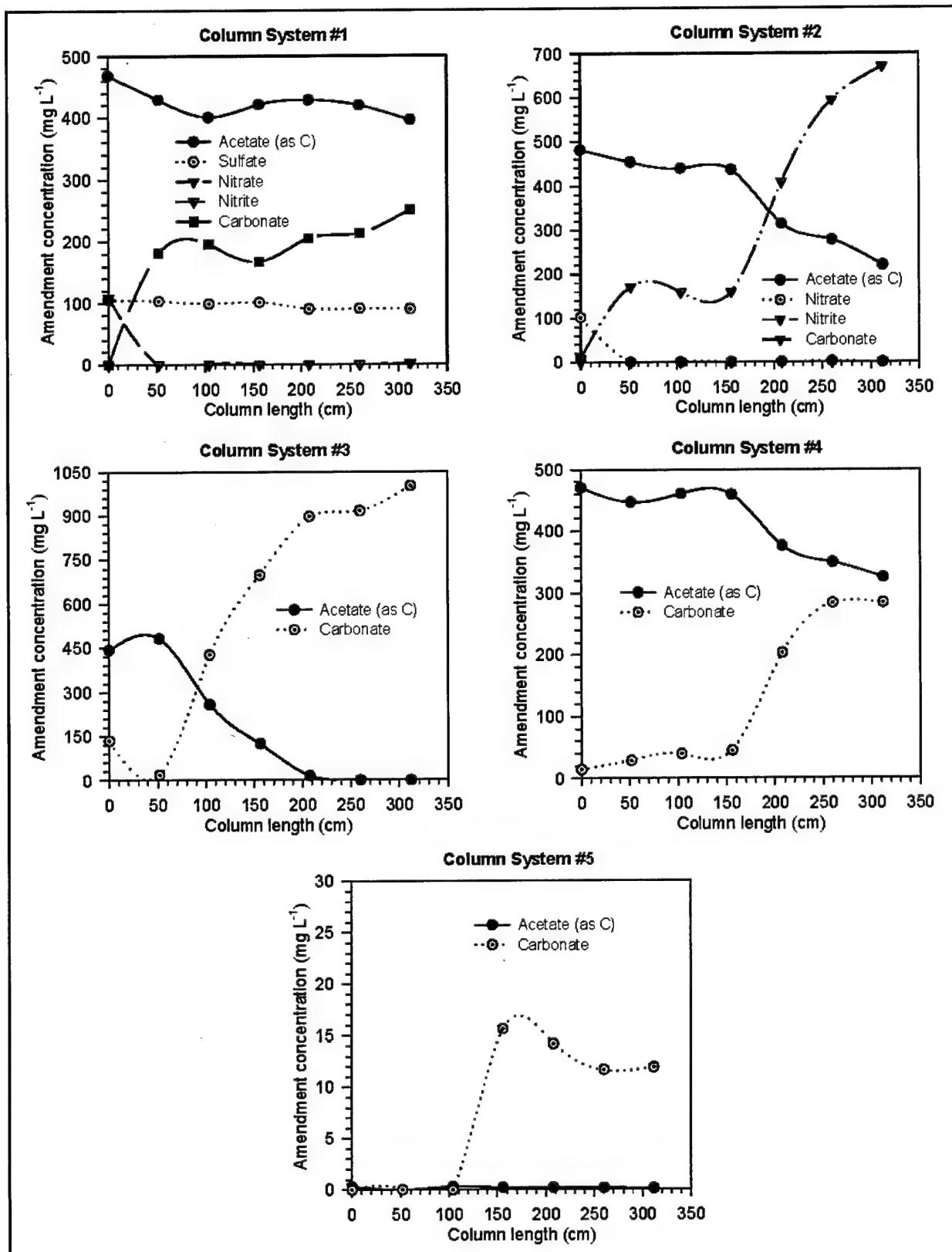


Figure 15. Axial concentration profile of amendments at Week 24 (Bed Profile #3)

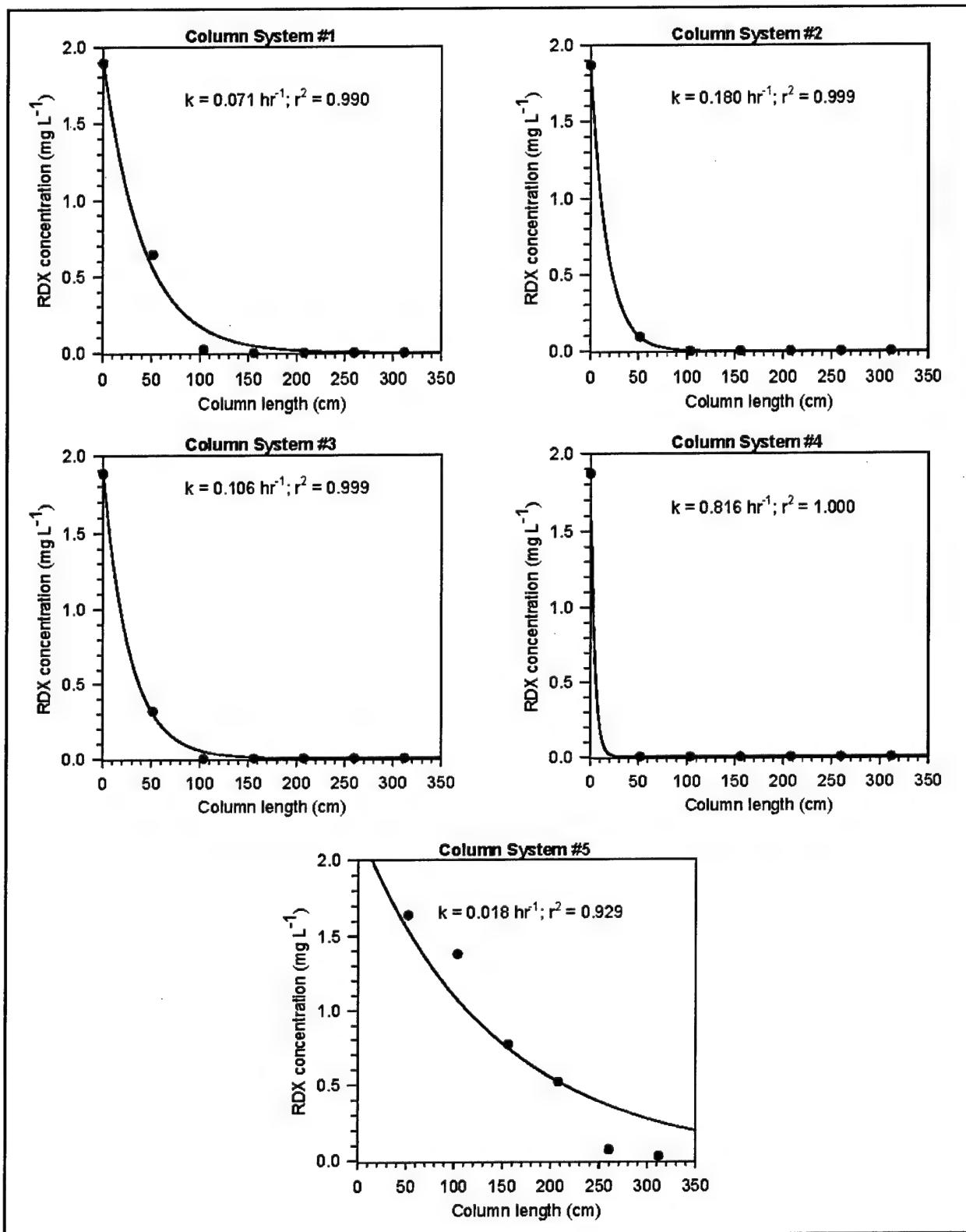


Figure 16. RDX biodegradation kinetics analysis at Week 12 (Bed Profile #1)

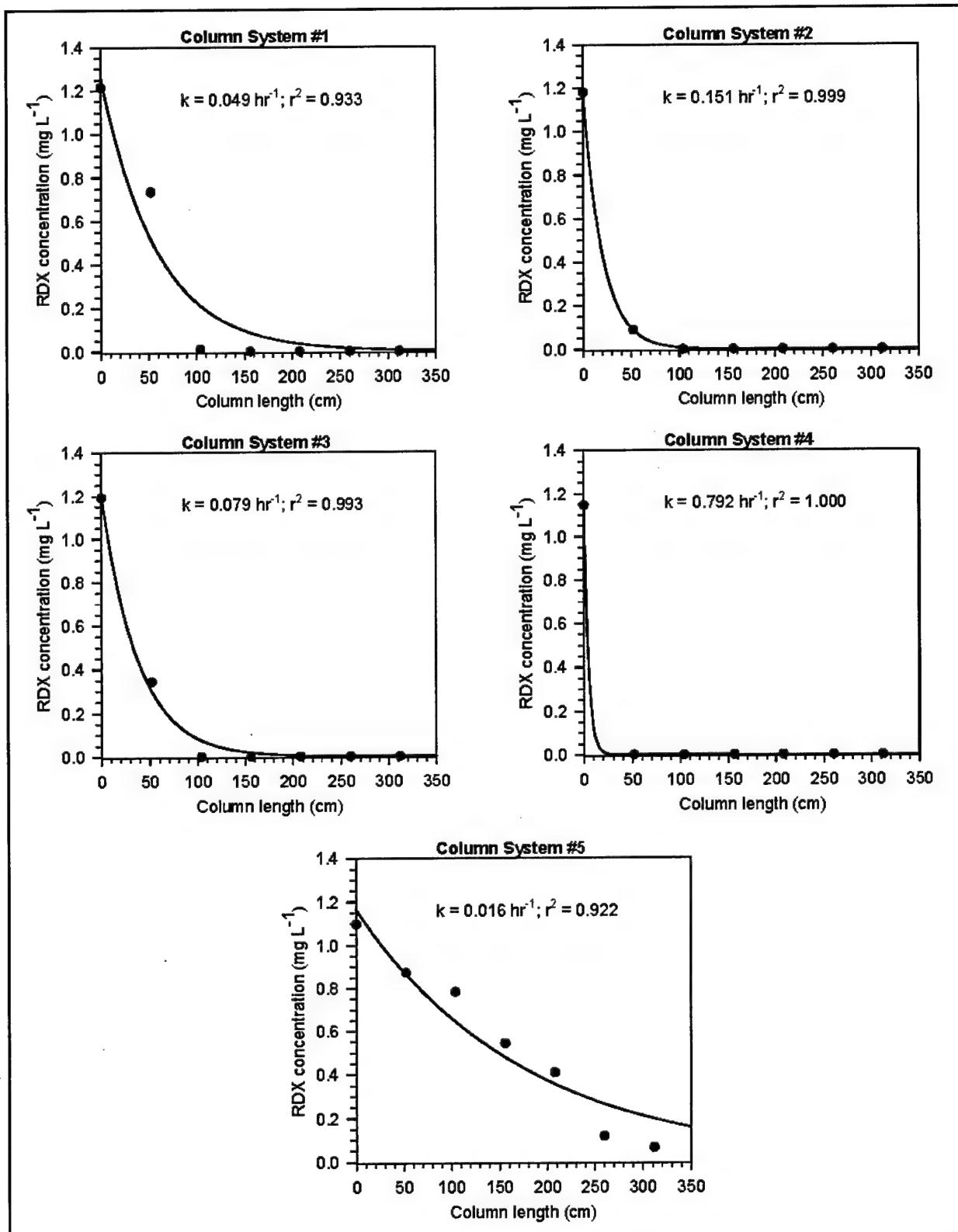


Figure 17. RDX biodegradation kinetics analysis at Week 18 (Bed Profile #2)

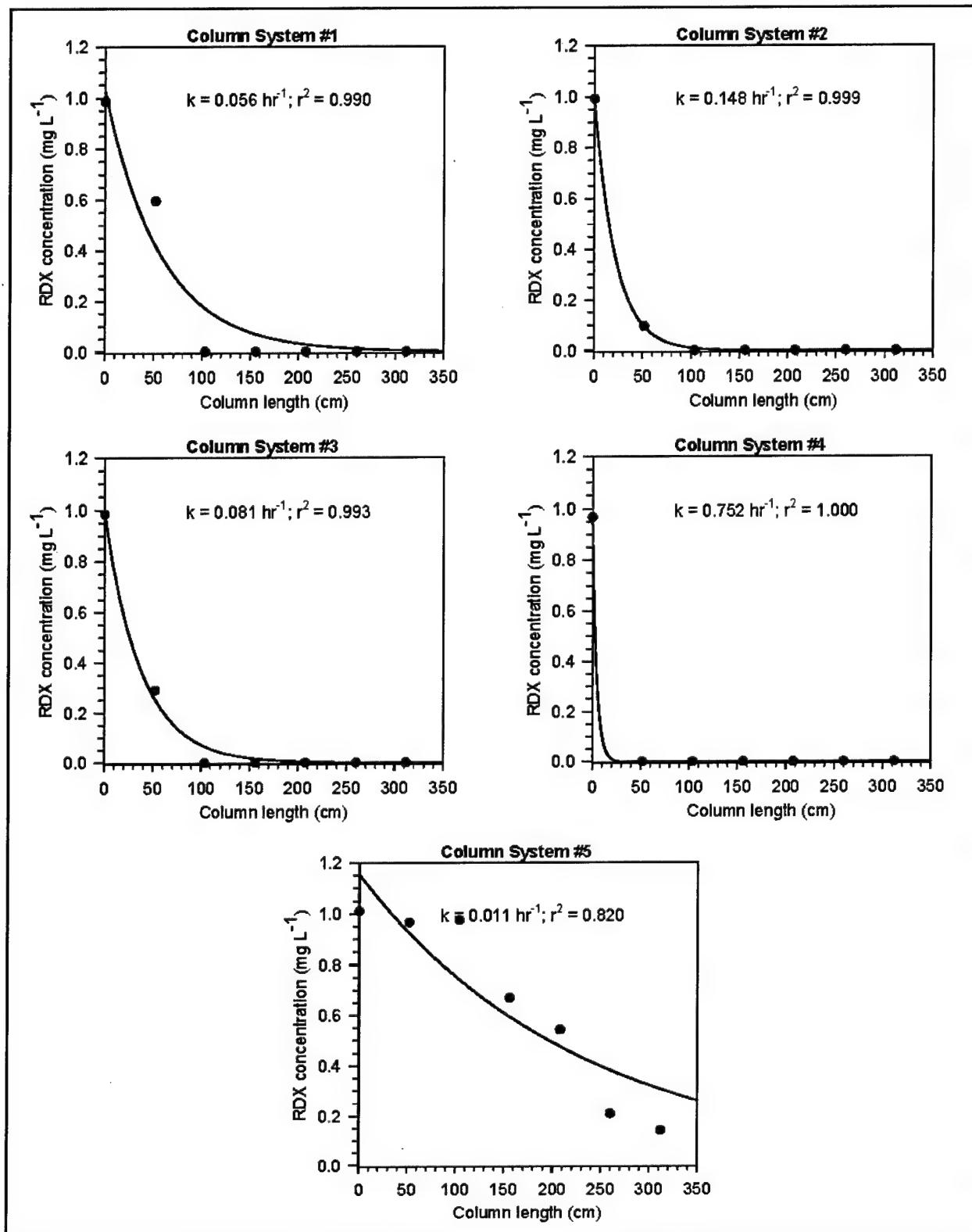


Figure 18. RDX biodegradation kinetics analysis at Week 24 (Bed Profile #3)

Table 3
RDX Biodegradation Rate Kinetics

Column System	Amendment Composition	Biodegradation Rate Coefficient k , hr^{-1}	Estimated Half-Life $t_{1/2}$, hr
1	Acetate (500); Sulfate (100); Nitrate (100); Ammonium (100)	0.059 ± 0.012	12.12 ± 2.28
2	Acetate (500); Nitrate (100); Ammonium (100)	0.160 ± 0.018	4.38 ± 0.46
3	Acetate (500); Ammonium (100)	0.088 ± 0.015	7.98 ± 1.24
4	Acetate (500)	0.787 ± 0.032	0.88 ± 0.04
5	No amendment	0.015 ± 0.004	47.65 ± 12.32
HNS	Acetate (500); Sulfate (100); Nitrate (500)	0.022	31.68
HN	Acetate (500); Nitrate (500)	0.020	34.31

Note: Values in parentheses are nominal concentrations as $mg\ L^{-1}$. Acetate concentration is presented as carbon. Standard deviation is calculated over three tests ($n = 3$).

biodegradation rate. In the presence of nitrate and sulfate, the RDX biodegradation rate coefficient was $0.06\ hr^{-1}$, about 13 times less than the one obtained in the absence of nitrate and sulfate. The presence of nitrate in addition to carbon source reduced the biodegradation rate coefficient by approximately 80 percent to $0.16\ hr^{-1}$. Contrary to expectations, the presence of ammonium in conjunction with acetate resulted in a lower ($0.09\ hr^{-1}$) biodegradation rate coefficient as compared to the one obtained in the absence of ammonium. Although Beller (2002) has reported that ammonium had no beneficial effects on RDX biodegradation, in this study the presence of ammonium caused adverse effects by delaying RDX biotransformation probably as a result of preferential use of ammonium as a N source.

Effects of High Nitrate Levels on RDX Biodegradation

Two tests were performed with high levels of nitrate ($500\ mg\ L^{-1}$) in conjunction with acetate ($500\ mg\ L^{-1}$) as the sole electron donor. In the first test, $100\ mg\ L^{-1}$ of sulfate was used in the amendment mixture, and in the second test no sulfate was used. The results of the first test are summarized in Figure 19. Initially RDX degradation was almost completely halted within the first 52 cm (1.7 ft) of column because of high levels of nitrate (Figures 19A and D). Nitrate was completely removed at the first sampling port irrespective of the presence of sulfate (Figures 19B and E). High levels of nitrite as a result of nitrate denitrification were observed throughout the column length beyond the first 52 cm (1.7 ft). As the nitrite levels reduced along the column length, RDX biodegradation followed the same pattern. No significant (95-percent confidence) differences in RDX biodegradation were observed in the presence (Figure 19A) or absence (Figure 19D) of sulfate. One notable observation in the presence of high levels of nitrate was that the RDX removal along the column length occurred without the detection of any nitroso-substituted transformation products. None of the influent sulfate ($100\ mg\ L^{-1}$) was used by the biological activity

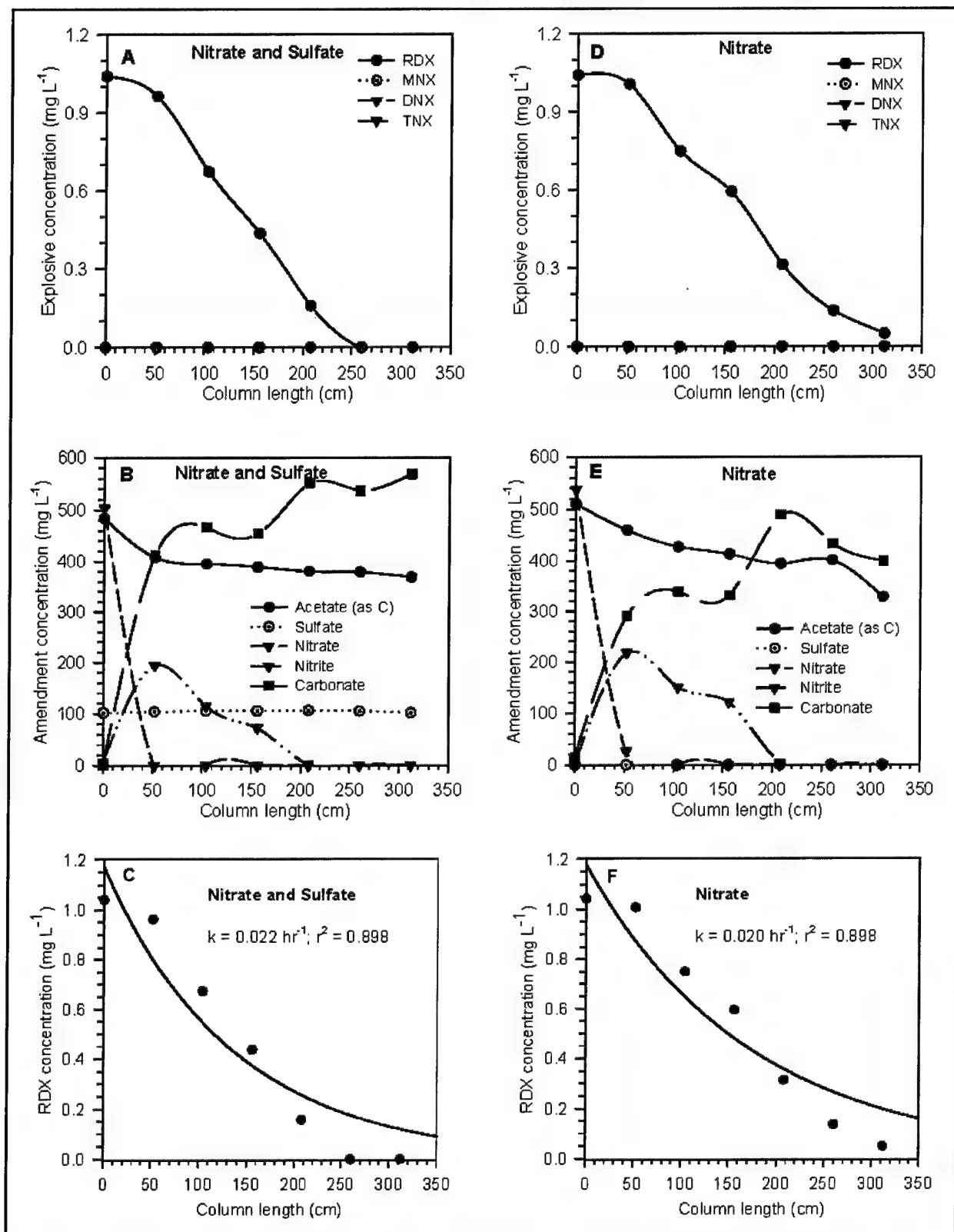


Figure 19. Effects of high nitrate levels on axial concentration profile of explosives, amendments, and rate of RDX biodegradation

(Figure 19B), perhaps due to the lack of sulfate-reducing microorganisms or as a result of inhibition from RDX transformation products. Although only about 20 percent of the influent acetate was used by the biological activity, high levels of carbonate were detected along the column length. Also, evident from Figures 19B and E is that primary acetate consumption occurred in the first 52 cm (1.7 ft) of the column system where nitrate denitrification occurred. After that, the acetate was used sparingly by the biological activity to denitrify nitrite in the remaining column length.

The kinetic analysis of RDX biodegradation in coexistence with high levels of nitrate is shown in Figures 19C and F. RDX first-order biodegradation rate coefficients (0.02 hr^{-1}) were similar in the presence or absence of sulfate. These nitrate concentrations reduced the rate coefficient obtained in the absence of nitrate (0.79 hr^{-1}) by about 98 percent to 0.02 hr^{-1} , thereby increasing the half-life to 32 hr. This rate coefficient was quite close to the rate coefficient obtained in the amendment-control column system (0.015 hr^{-1}). These findings demonstrate the adverse effects of high levels of nitrate on reductive transformation of RDX and highlight the need for sufficient quantities of a carbon source to quench the denitrification demands. These results corroborate the earlier findings that RDX biotransformation is temporarily inhibited by the presence of nitrate (Freedman and Sutherland 1998; Beller 2002; Bhushan et al. 2002).

6 Conclusions

The column study reported herein provided several elements of useful information on reductive biotransformation of RDX in groundwater in the presence of inorganic electron acceptors such as nitrate and sulfate.

Influent RDX concentrations were removed without the detection of any nitroso derivatives in amendment-treatment columns while low levels of nitroso metabolites were observed in amendment-control columns. The presence of different transformation products under amendment-treatment and amendment-control conditions demonstrates that the fate of RDX is highly dependent on redox conditions. Under very low redox conditions, nitroso-substituted transformation products seem to be unstable and undergo further transformation, perhaps the ring cleavage, as proposed by previous researchers. On the other hand, under moderately reduced conditions these nitroso-transformation products persist and accumulate.

The results of this study demonstrated that biotransformation of RDX is inhibited under high redox conditions and in the presence of high nitrate levels. High nitrate concentrations appeared to inhibit the RDX biodegradation, thereby delaying RDX biotransformation. Low levels of nitrate and sulfate did not halt the RDX biotransformation, but did reduce the RDX biodegradation rate coefficient. Low redox conditions can be achieved by providing sufficient quantities of a readily biodegradable carbon source such as acetate. The carbon source (electron donor) provided should be in excess of denitrification demands to quench demands of other electron acceptors and oxidants. Furthermore, to avoid the accumulation of more toxic nitroso-substituted metabolites, and to achieve complete transformation of RDX and its nitroso derivatives, a very low redox environment with no or low levels of nitrate is needed.

References

Agency for Toxic Substances and Disease Registry. (1996). "TOX FAQsTM for RDX," <http://www.atsdr.cdc.gov/tfacts78.html> (accessed March 5, 2003).

Bayman, P., Ritchey, S. D., and Bennett, J. W. (1995). "Fungal interactions with the explosive RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)," *J. Indus. Microbiol.* 15, 418-423.

Beller, H. R. (2002). "Anaerobic biotransformation of RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) by aquifer bacteria using hydrogen as the sole electron donor," *Water Res.* 36, 2533-2540.

Beller, H. R., and Tiemeier, K. (2002). "Use of liquid chromatography/tandem mass spectrometry to detect distinctive indicators of in situ RDX transformation in contaminated groundwater," *Environ. Sci. Technol.* 36, 2060-2066.

Bhushan, B., Halasz, A., Spain, J., Thiboutot, S., Ampleman, G., and Hawari, J. (2002). "Biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine catalyzed by a NAD(P)H: Nitrate oxidoreductase from *Aspergillus niger*," *Environ. Sci. Technol.* 36: 3104-3108.

Binks, P. R., Nicklin, S., and Bruce, N. C. (1995). "Degradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by *Stenotrophomonas maltophilia* PB1," *Appl. Environ. Microbiol.* 61, 1318-1322.

Brenner, A., Ronen, Z., Harel, Y., and Abeliovich, A. (2000). "Use of hexahydro-1,3,5-trinitro-1,3,5-triazine as a nitrogen source in biological treatment of munitions wastes," *Water Environ. Res.* 72, 469-475.

Coleman, N. V., Nelson, D. R., and Duxbury, T. (1998). "Aerobic biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) as a nitrogen source by a *Rhodococcus* sp. strain DN22," *Soil Biol. Biochem.* 30, 1159-1167.

Defense Environmental Network and Information Exchange. (2002). "Enhanced alternative and in situ treatment technologies for explosives in groundwater," <http://www.denix.osd.mil/denix/DOD/Policy/Army/Aerta/Report/a12a.html> (accessed March 5, 2003).

Etiner, E. L. (1989). "Water quality criteria for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)," *Regulatory Toxicol. Pharmacol.* 9, 147-157.

Fellows, R. J., Harvey, S. D., and Cataldo, D. A. (1995). "Evaluation of the metabolic fate of munitions material (TNT & RDX) in plant systems and initial assessment of material interaction with plant genetic material. Validation of the metabolic fate of munitions material (TNT & RDX) in mature crops," PNL-10825, U.S. Army Biomedical Research and Development Laboratory, Fort Detrick, Fredrick, MD.

Fernando, T., and Aust, S. D. (1991). "Biodegradation of munition waste, TNT (2,4,6, trinitrotoluene) and RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)," *Emerging technologies in hazardous waste management*. D. W. Tedder and F. G. Pohland, ed., American Chemical Society, Washington, DC, 214-231.

Freedman, D. L., and Sutherland, K. W. (1998). "Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) under nitrate-reducing conditions," *Wat. Sci. Tech.* 38, 33-40.

Halasz, A., Spain, J., Paquet, L., Beaulieu, C., and Hawari, J. (2002). "Insights into the formation and degradation mechanisms of methylenedinitramine during the incubation of RDX with anaerobic sludge," *Environ. Sci. Technol.* 36, 633-638.

Harvey, S. D., Fellows, R. J., Cataldo, D. A., and Bean, R. M. (1991). "Fate of the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in soil and bioaccumulation in bush bean hydroponic plants," *Env. Toxic. & Chem.* 10, 845- 855.

Hawari, J., Halasz, A., Sheremata, T., Beaudet, S., Groom, C., Paquet, L., Rhofir, C., Ampleman, G., and Thiboutot, S. (2000). "Characterization of metabolites during biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) with municipal anaerobic sludge," *Appl. Environ. Microbiol.* 66, 2652-2657.

He, J., Sung, Y., Dollhopf, M. E., Fathepure, B. Z., Tiedje, J. M., and Loffler, F. E. (2002). "Acetate versus hydrogen as direct electron donor to stimulate the microbial reductive dechlorination process at chloroethene-contaminated sites," *Environ. Sci. Technol.* 36, 3945-3952.

Kitts, C. L., Cunningham, D. P., and Unkefer, P. J. (1994). "Isolation of three hexahydro-1,3,5-trinitro-1,3,5-triazine degrading species of the family *Enterobacteriaceae* from nitramine explosive-contaminated soil," *Appl. Environ. Microbiol.* 60, 4608-4711.

Larson, S. L., Jones, R. P., Escalon, L., and Parker, D. (1999). "Classification of explosives transformation products in plant tissue," *Env. Tox and Chem.* 18, 1270-1276.

McCormick, N. G., Cornell, J. H., and Kaplan, A. M. (1981). "Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine," *Appl. Environ. Microbiol.* 42, 817-823.

Morley, M. C., Shamma, S. N., and Speitel G. E., Jr. (2002). "Biodegradation of RDX and HMX mixtures: Batch screening experiments and sequencing batch reactors," *Environ. Eng. Sci.* 19, 237-250.

Oh, B-T., Just, C. L., and Alvarez, P. J. J. (2001). "Hexahydro-1,3,5-trinitro-1,3,5-triazine mineralization by zerovalent iron and mixed anaerobic cultures," *Environ. Sci. Technol.* 35, 4341-4346.

Pennington, J. C. (1999). "Explosives." *Environmental availability of chlorinated organics, explosives, and metals in soils*. W. C. Anderson, R. C. Loehr, B. P. Smith, ed., American Academy of Environmental Engineers, Annapolis, MD, 85-109.

Sheremata, T. W., and Hawari, J. (2000). "Biodegradation of RDX by the white rot fungus *Phanerochaete chrysosporium* to carbon dioxide and nitrous oxide," *Environ. Sci. Technol.* 34, 3384-3388.

Sheremata, T. W., Halasz, A., Paquet, L., Thiboutot, S., Ampleman, G., and Hawari, J. (2001). "The fate of the cyclic nitramine explosive RDX in natural soil," *Environ. Sci. Technol.* 35, 1037-1040.

Singh, J., Comfort, S. D., Hundal, L. S., and Shea, P. J. (1998). "Long-term RDX sorption and fate in soil," *J. Environ. Qual.* 27, 572-577.

Thompson, P. L., Ramer, L. A., and Schnoor, J. L. (1999). "Hexahydro-1,3,5-trinitro-1,3,5-triazine translocation in popular trees," *Env. Tox. and Chem.* 18, 279-284.

U.S. Army. (1984). "Military explosives," TM 9-1300-214, Washington, DC.

U.S. Environmental Protection Agency. (1994). "Nitroaromatics and nitramines by high performance liquid chromatography (HPLC)," SW-846, Method 8330, Rev. 4. Washington, DC.

_____. (2002). "Drinking water standards and health advisories," EPA 822-R-02-038, Office of Water, Washington, DC.

Wani, A. H., O'Neal, B. R., Davis, J. L., and Hansen, L. D. (2002). "Treatability study for biologically active zone enhancement (BAZE) for in situ RDX degradation in groundwater," ERDC/EL TR-02-35, U.S. Army Engineer Research and Development Center, Vicksburg, MS.

Young, D. M., Unkefer, P. J., and Ogden, K. L. (1997) "Biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by a prospective consortium and its most effective isolate *Serratia marcescens*," *Biotechnol. Bioeng.* 53, 515-522.

14. (Concluded)

High levels of nitrate (500 mg L⁻¹) initially halted RDX removal and significantly reduced the rate of RDX biotransformation by about 98 percent to 0.02 hr⁻¹, thereby increasing the half-life from 0.9 hr in the absence of nitrate to about 32 hr, with noticeable levels of untreated RDX in the effluent stream. Contrary to expectations, presence of ammonium in conjunction with acetate resulted in a lower (0.09 hr⁻¹) biodegradation rate coefficient as compared to the one obtained in absence of ammonium.